Limitations and trainability of the respiratory system during exercise with thoracic loads

UNIVERSITY OF DERBY

LIMITATIONS AND TRAINABILITY OF THE RESPIRATORY SYSTEM DURING EXERCISE WITH THORACIC LOADS

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ORAL COMMUNICATIONS

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Mann, J., **Faghy, M, A.,** (2015), Does inspiratory muscle training attenuate reductions in maximum voluntary contraction of the quadriceps after load carriage?, Oral presentation at the annual British Association of Sport and Exercise Sciences Student Conference, Liverpool John Moors University, UK.

Faghy, M, A., Brown, P, I., (2014), Training the inspiratory muscles improves running performance when carrying a 25 kg thoracic load in a backpack, Oral presentation at the annual British Association of Sport and Exercise Sciences, St Georges Park, Burton Upon Trent, UK.

Faghy, M, A., Brown, P, I., (2013), Thoracic load carriage induced respiratory muscle fatigue, Oral presentation at the annual British Association of Sport and Exercise Sciences Student Conference, Cardiff Metropolitan University, UK. **Winner of Post-graduate oral presentation award.**

Faghy, M, A., Brown, P, I., (2013), Implications of thoracic load carriage on respiratory function, Oral presentation at the University of Derby annual New Horizons conference, University of Derby, UK. **Winner of best abstract award.**

POSTER COMMUNICATIONS

Lester, P., **Faghy, M, A.,** (2015), Inspiratory Muscle Function during Preloaded Time Trial Performance using Traditional (IMT_{TRAD}) and Functional (IMT_{FUNC}) Inspiratory Muscle Training (IMT). Poster presentation at the annual British Association of Sport and Exercise Sciences Student Conference, Liverpool John Moors University, UK.

Sharp, C., **Faghy, M. A.,** (2014), The Effect of IMT on Cycling Time-Trial Performance during COOL (~16°C) and HOT (~26°C) Conditions. Poster Presentation presentation at the annual British Association of Sport and Exercise Sciences Student Conference, Portsmouth University, UK.

Farley, K., **Faghy, M. A.,** (2014), Effects of Inspiratory Muscle Training on Physiological Mechanisms and responses, involving mixed martial arts based exercises. Poster Presentation presentation at the annual British Association of Sport and Exercise Sciences Student Conference, Portsmouth University, UK.

LIST OF ABBREVIATIONS

ACT	Active warm-up trial
ACT + IMWU	Combined active and inspiratory muscle warm-up trial
ACT+PLA	Combined active and placebo warm-up trial
ANOVA	Analysis of variance
$[lac^-]_B$	Blood lactate concentration
BPNS	Bi-lateral phrenic nerve stimulation
CO_2	Carbon dioxide
CON	Unloaded trials
CON_{TT}	Control time trial
CV	Coefficient of variation
DC_{ExP}	Expired duty cycle
EELV	End expiratory lung volume
EILV	End inspiratory lung volume
EMG	Electromyography
EMT	Expiratory muscle training
f_B	Breathing frequency
FEV_1	Forced expiratory volume in one second
FEV_1/ FVC (%)	Ratio of forced expiratory volume in one second and forced vital capacity
F_IO_2	Fractional concentration of inspired oxygen
FRC	Functional residual capacity
FRL	Flow resistive loading
FVC	Forced vital capacity
$[glucose]_B$ -	Blood glucose concentration
$[lac]_B$ -	Blood lactate concentration
HFF	High frequency fatigue
HR	Heart rate
H_Z	Hertz

IMT	Inspiratory muscle training
IMT_{CON}	Maintenance of inspiratory muscle training
IMT_F	Functional inspiratory muscle training
IMWU	Inspiratory muscle warm-up trial
lac^-	Lactate
LC	Load carriage trial
LC_{TT}	Load carriage time trial
LFF	Low frequency fatigue
LoA	Limits of agreement
MNSA	Muscle sympathetic nerve activity
MVC	Cardiac output
O_2	Oxygen
MAP	Mean arterial pressure
PAV	Proportional assist ventilator
P_{di}	Trans-diaphragmatic pressure
P_{gas}	Gastric pressure
P_{oes}	Oesophageal pressure
Ppl	Pleural pressure
PEF	Peak expiratory flow
P_aCO_2	Arterial pressure of carbon dioxide
P_{Emax}	Maximal expiratory pressure
P_{Imax}	Maximal inspiratory pressure
PLA	Placebo
PTL	Pressure threshold loading
$\dot{Q}$	Cardiac output
RER	Respiratory exchange ratio
RPE	Rate of perceived exertion
$RPE_{breathing}$	Rate of perceived exertion specific to breathing

RPE_{legs}	Rate of perceived exertion specific to leg discomfort
RMT	Respiratory muscle training
RV	Residual volume
SCBA	Self-contained breathing apparatus
SD	Standard deviation
SMIP	Sustained maximal inspiratory pressure
STPD	Standard temperature, pressure and dry
SV	Stroke volume
TIRE	Test of incremental respiratory endurance
TLC	Total lung capacity
T_E	Expiratory time
T_I	Inspiratory time
T_{TOT}	Total breath time
V_A	Alveolar ventilation
VA	Voluntary activation
VC	Vital capacity
$\dot{V}CO_2$	Carbon dioxide production
$\dot{V}_E$	Minute ventilation
VIH	Voluntary isocapnic hyperpnoea
VLTP	Velocity at Lactate Turnpoint
$\dot{V}O_2$	Oxygen uptake
$\dot{V}O_{2peak}$	Maximal oxygen uptake
V_T	Tidal volume
WOB	Work of breathing

ABSTRACT

Thoracic loads (i.e., a heavy backpack) commonly used in occupational and recreational settings significantly challenge human physiological systems and increase the work of breathing, which may promote respiratory muscle fatigue and negatively impacts whole body performance during physical tasks. Accordingly this thesis: (Chapter number: II) designed a laboratory based protocol that closely reflects occupational demands and (III) assessed the effect that load carriage (LC) has upon physiological and respiratory muscle function. Consequently the thesis addressed (IV) acute, (V) chronic and (VI) functional inspiratory muscle loading strategies to assess the limitations and trainability of the respiratory muscles to load carriage performance.

The novel laboratory protocol, performed wearing a 25 kg backpack load, combined submaximal load carriage (LC; 60 min treadmill march at 6.5 $km \cdot h^{-1}$) and self-paced time trial exercise (LC_{TT}; 2.4 km) to better reflect the physiological demands of occupational performance (between trials mean difference -0.34 ± 0.89 min, coefficient of variation 10.5%). Following LC, maximal inspiratory muscle pressure (P_{Imax}) and maximal expiratory muscle pressure (P_{Emax}) were reduced by 11% and 13% respectively ($P<0.05$), and further by 5% and 6%, respectively ($P< 0.05$), after LC_{TT}. Acute inspiratory loading (2 × 30 forced inspiratory efforts 40% P_{Imax}) following an active warm-up (10 min lactate turnpoint) failed to improve LC_{TT} despite a transient increase in P_{Imax} of ~7% ($P<0.05$). Chronic inspiratory loading (6 wk, 50% P_{Imax}, 30 breaths twice daily) increased P_{Imax} (31%, $p<0.05$) reduced HR and perceptual responses post-LC, and improved LC_{TT} (8%, $P< 0.05$) with no change in a placebo control. Combining IMT with functional core muscle exercises improved P_{Imax} and LC_{TT} by 7% and 4% respectively ($P< 0.05$), which was greater than traditional IMT alone. Acute, chronic and functional inspiratory muscle loading strategies did not protect against respiratory muscle or locomotor muscle fatigue during LC and LC_{TT}.

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CHAPTER 1

GENERAL INTRODUCTION AND LITERATURE REVIEW

1.1 THE RESPIRATORY SYSTEM

The human body is inadequate of self-sustaining all essential bodily processes; pulmonary ventilation is one system that is an essential component that coincides and interacts with other visceral systems (e.g. cardiovascular) to sustain life. The respiratory system is primarily responsible for providing alveolar ventilation (V_A), with primary tasks being the preservation of the partial pressure of arterial blood gases oxygen (O_2) and carbon dioxide (CO_2), and sustaining acid-base balance (Sheel & Romer, 2012). The onset of exercise increases both the consumption of O_2 and the production of CO_2, which in large quantities is problematic causing respiratory acidosis and must therefore be expelled. Maintaining respiratory gas concentrations is an imperative task that is achieved with remarkable precision and with minimal energy cost to the body. This requires precise alterations stemming from a series of complex interactions between both central and neural processes (Butler, 2007), that occurs in proportion to increased metabolic demand. Such changes occur with minimal energetic cost as to sustain homeostasis (Dempsey, Romer, Rodman, Miller, & Smith, 2006; Dominelli & Sheel, 2012), and occurs through the co-ordination of the lung parenchyma, airways, respiratory control systems and respiratory muscles.

1.1.1 ANATOMY OF THE BREATHING SYSTEM

The respiratory system and thorax comprises of two main anatomical components; i) the lung parenchyma, which is responsible for continuous exchange of respiratory gases, and ii) the respiratory muscles, which provide a ventilatory pump and are recruited sequentially to assist with increased ventilation during exercise. Although the upper respiratory tract is not the focus of this thesis, brief detail is provided to summarise its structure and function which are essential for a holistic understanding of the pulmonary system. Consisting of the oral,

nasal cavities, larynx and the proximal end trachea, the upper respiratory tract is the first site in the body where environmental air enters the body via the oral and nasal cavities, and passes through the trachea into the lower respiratory tract, which has greater relevance for this thesis.

1.1.1.1 THE LOWER RESPIRATORY TRACT

The lower or distal respiratory tract (depicted in Figure 1.1) consists of the distal end of the trachea and the superior bronchi and is the first of 23 partitions that occur within the airway that initially separate into the left and right bronchi (McConnell, 2011). The airways during each of the partitions become narrower in order to create a larger surface area within the lung (50 to 100 m^2); where at the very distal end of the airway small air sacs called alveoli are located. Within the lung there are approximately ~500 million alveoli and is the site of passive diffusion, where alveoli gasses transfer into a dense network of capillaries that surround the alveoli (West, 2000).

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See the following reference for full image:

Griesenbach, U., Alton, E. W., Consortium, U. C. F. G. T., & others. (2009). Gene transfer to the lung: lessons learned from more than 2 decades of CF gene therapy. Advanced Drug Delivery Reviews, 61(2), 128–139.

Figure 1.1 Lower respiratory tract that comprises the lung and airways which terminates at the alveoli, the site of passive diffusion (adapted from Griesenbach, Alton, Consortium, & others, 2009).

1.1.1.2 THE THORAX

The lower respiratory tract is contained within a semi-rigid, elastic cage like structure known as the thorax (Figure 1.2). This houses sites of attachment for the obligatory and accessory respiratory musculature (Outlined in Section 1.1.2) which permit either positive or negative changes in intra-thoracic pressure and volume when under contraction (Cappello & De Troyer, 2002). This is assisted by the pleural cavity which exists proximal to the thoracic cavity and between two membranous (visceral and outer parietal) layers that line both the lungs and the thoracic cavity. Contained within this space, pleural fluid acts as a lubricant that facilitates pleurae displacement during ventilation. Conduction of intra-thoracic pressures occurs here, permitting lung expansion as the chest wall transmits pressures to the visceral pleura via the lung (Lai-Fook, 2004). Other anatomical features include twelve pairs of ribs which attach to the sternum anteriorly and also articulate posteriorly with the thoracic vertebrae, known as costochondral junction (De Troyer, Kirkwood, & Wilson, 2005). The first rib is comparatively flat and articulates with the manubrium sterni in an immovable cartilaginous joint. Ribs two to ten are relatively fixed at their origins (costovertebral and costotransverse joints) during the breathing cycle; so rotation occurs through long axis of its neck (De Troyer et al., 2005). Ribs eleven and twelve are primarily known as floating ribs and interact directly with the inner surface of the abdominal wall.

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See the following reference for full image:

Stone, J., & Stone, R. (2011). Atlas of Skeletal Muscles. McGraw-Hill Education.

Figure 1.2 Diagrammatic representation of the thorax, (Image adapted from Stone & Stone, 2011).

Variation in the displacement of individual ribs within the thorax exists to assist with increasing thoracic volume. The upper ribs (1-4) are more rigid due to the insertion onto the sternum and displace cranially with the sternum during inspiration, causing increased thoracic volume and surface area (De Troyer, 2012); due to what is known as the pump handle action (Figure 1.3, panel A). The lower ribs displace differently as isolated contractions of the diaphragm during inspiration induces cranial and outward displacement (De Troyer, 2012), known as the bucket handle action and calliper action (Figure 1.3, panels B,C).

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See the following reference for full image:

De Troyer, A., Kirkwood, P. A., & Wilson, T. A. (2005). Respiratory action of the intercostal muscles. Physiological Reviews, 85(2), 717–756.

Figure 1.3 Displacement action of the ribs during inspiration A) pump handle action of the upper ribs; B) bucket handle motion of the middle ribs and C) calliper-like motion of the lower ribs (De Troyer et al., 2005).

1.1.2 RESPIRATORY MUSCULATURE

As previously described the respiratory musculature insert in various locations onto the thoracic cavity which is detailed specifically in later sections. When under contraction, these muscles alter the dimensions and intra-thoracic pressures, permitting flow and assisting with ventilation (Hudson, Gandevia, & Butler, 2011). Although they are termed respiratory muscles due their role in ventilation, some respiratory muscles are tasked with multiple roles which may include; locomotion, stability, and the production of speech (Aleksandrova & Breslav, 2009), which is an important concept for this thesis. Ventilation can be adequately devised into two distinct phases; inspiration and expiration of which the muscles responsible are further classified as either obligatory muscles of respiration which contract throughout every breath during quiet breathing. The muscles that are recruited additionally and contract when ventilation is increased are termed accessory muscles of respiration (Hudson et al., 2011).

1.1.2.1 MUSCLES OF INSPIRATION

Inspiration occurs as a direct result of a contraction of the inspiratory muscles which brings about an expansion of the chest cavity and subsequently alters pleural and atmospheric pressures within the thoracic cavity. The pressure changes cause alveolar pressure to become sub-atmospheric, inducing airflow into the lungs (Sheel, 2002). Inspiration is led by the obligatory muscles which include the diaphragm, parasternal intercostal, scalenes and the external intercostals (Ratnovsky et al., 2008), which are continuously active throughout respiration.

1.1.2.1.1 DIAPHRAGM

The diaphragm (Figure 1.4) works continuously throughout human life and is a vital tool in ventilation. It is a dome shaped sheet of muscle that separates both the thoracic and abdominal cavities (Ellis, 2008a), which during inspiration moves downwards increasing thoracic volume. Mean diaphragm mass was obtained from a cadaver study and represented on average 0.5% of body mass (283 ± 53 g) from a sample ranging from 16-91 years (Rochester, Arora, & Braun, 1982). The diaphragm is solely innervated by the phrenic nerves (C3, C4 and C5) to provide the motor supply for the diaphragm (Ellis, 2008a), which attach to the 7-12th ribs (Aliverti, 2008a) and importantly is one of few muscles to insert directly on to the lumbar and thoracic regions of the spine (Hodges, Eriksson, Shirley, & Gandevia, 2005). The muscle fibres of the diaphragm originate from the central tendon to the diaphragms two sections, the costal and the crural regions (Ratnovsky, Elad, & Halpern, 2008). The costal region originates from the third, fourth and fifth cervical segments and is responsible for contracting and generating a caudal force on the central tendon, which as a result descends to expand the thoracic cavity. In addition to this, the costal fibres apply a force on the lower six ribs which lifts and rotates them outward (Ratnovsky et al., 2008). The crural region originates from the mesentery of the oesophagus; both regions have distinct differences in

their mechanical and physiological functions in particular during inspiration. Here, the costal and crural regions have differing mechanical properties that affect the rib cage (Sharshar et al., 2005). Firstly, when under contraction the costal diaphragm results in expansion of the rib cage and abdomen whereas the crural diaphragm has less power to cause rib cage expansion and does not cause any expansion of the lower ribcage (De Troyer, Sampson, Sigrist, & Macklem, 1982). Secondly, the crural region has preferential involvement in the controlled relaxation of the diaphragm to assist with expiration, demonstrated by a longer contraction duration and an increased post-inspiratory activity (Sharshar et al., 2005). There are also notable differences in the contribution to postural stability, the costal region ensures rib cage stability while the crural region increases spinal stability (Hodges, Heijnen, & Gandevia, 2001).

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See the following reference for full image:

Rochester, D. F., Arora, N. S., & Braun, N. M. (1982). Maximum contractile force of human diaphragm muscle, determined in vivo. Transactions of the American Clinical and Climatological Association, 93, 200–208.

Figure 1.4 Schematic representation of the human diaphragm at functional residual capacity (adapted from Rochester et al., 1982).

1.1.2.1.2 SCALENES

The scalene group originate at the lateral aspect of the neck and exists as three muscles; the anterior, medial and posterior heads (Figure 1.5, Olinger & Homier, 2010). The anterior scalene originates from vertebrae C3–C6 and inserts on scalene tubercle of the first rib, the

medial scalene originates from C2-C7 and also inserts on the first rib, and the posterior scalene originates from C4, C5 and C6 vertebrae and inserts onto the second rib (Olinger & Homier, 2010). The scalene muscles, which as previously detailed pass from the cervical vertebrae to the first rib, are responsible for cranial displacement of the thorax and surrounding muscles of the limb girdles, including the pectorals, latissimus dorsi and serratus anterior (Ellis, 2008b; Hudson, Gandevia, & Butler, 2007).

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See the following reference for full image:

Olinger, A. B., & Homier, P. (2010). Functional anatomy of human scalene musculature: rotation of the cervical spine. Journal of Manipulative and Physiological Therapeutics, 33(8), 594–602.

Figure 1.5 Illustration of the lateral aspect of the cervical region representing the origin and insertion of the anterior scalene, medial scalene and posterior scalene muscles. (Adapted from Olinger & Homier, 2010).

Traditionally, the scalene muscle group were disregarded as an obligatory muscle of inspiration due to observed increases in activation with increased ventilatory demand (Guenette & Sheel, 2007; Ratnovsky et al., 2008). Research however has extensively highlighted, via the use of tomographic scanning and electromyography (EMG), that they do in fact contract in synchrony with the diaphragm (Aleksandrova & Breslav, 2009; Legrand, Schneider, Gevenois, & De Troyer, 2003) and experience greater activation during quiet breathing efforts compared to maximal inspiratory manoeuvres (Hudson et al., 2007).

1.1.2.1.3 PARASTERNAL INTERCOSTALS

The parasternal intercostals (Figure 1.6) are termed obligatory and are tasked with elevating the ribs enabling ait to flow into the lungs (Ratnovsky et al., 2008) which occurs as the parasternal intercostals contract. Contraction here causes rotation in synchrony with the scalenes and the diaphragm to occur at the costochondral junction ultimately elevating the ribs (De Troyer & Wilson, 2000).

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See the following reference for full image:

De Troyer, A., & Estenne, M. (1988). Functional anatomy of the respiratory muscles. Clinics in Chest Medicine, 9(2), 175–193.

Figure 1.6 Schematic representation of the parasternal intercostals that are located ventrally between the lateral borders of the sternum and the costochondral junctions (De Troyer & Estenne, 1988).

1.1.2.1.4 ACCESSORY MUSCLES OF INSPIRATION

The accessory muscles are made up of several thoracic, cervical and dorsal muscles and during times of peak ventilation are recruited sequentially to assist with inspiration (Aleksandrova & Breslav, 2009; Hudson et al., 2011). Research demonstrates through the use of volitional measurement techniques that changes in trans-diaphragmatic pressure (P_{di}) tend to plateau despite marked increases in minute ventilation ($\dot{V}_E$). This is indicative of reduced contribution, but not activation of the diaphragm during inspiration (Guenette & Sheel, 2007). This is aided by recruitment of a pool of accessory inspiratory muscles (Sheel, 2002), which

assist with ventilatory challenges and minimise the metabolic cost of ventilation during exercise (Sheel & Romer, 2012). Of importance here is that the accessory inspiratory muscles have dual roles that combine respiratory duties with other bodily functions such as movement and stabilisation of the torso and upper extremities (Aleksandrova & Breslav, 2009; Sheel & Romer, 2012). The accessory inspiratory musculature include the; sternocleidomastoid, levator costae and the intercostal muscles (Ratnovsky et al., 2008).

The sternocleidomastoid (Figure 1.7) run parallel to the scalene muscles and have an obligatory role of permitting the majority of head movements, and serves as an accessory muscle to the scalene musculature by providing cranial displacement of the upper thoracic region (De Troyer & Estenne, 1988; Shadgan, Guenette, Sheel, & Reid, 2011). Research has identified that activation of the sternocleidomastoid occurs progressively during incremental static and dynamic manoeuvres to maximal inspiratory pressure in healthy individuals (Butler & Gandevia, 2008), although occurring after the recruitment of the scalene (Hudson et al., 2007).

Figure 1.7 An anterior view of the sternocleidomastoids (adapted from Stone & Stone, 2011).

The external intercostal muscles are thin layers of muscle fibres that run downwards and ventrally from each rib to the neighbouring rib below (De Troyer et al., 2005; Ratnovsky et al., 2008) and when under contraction elevate the ribs and expand the thoracic cavity (Cappello & De Troyer, 2002). This occurs due to the lower insertion of the external intercostals muscles, which is distal from the ribs axis of rotation than the upper insertion. Consequently this exerts a larger torque acting on the lower rib which subsequently raises the lower rib with respect to the upper rib; the net effect of this causing elevation of the rib cage (Ratnovsky et al., 2008). Important to note here is that Wilson, Legrand, Gevenois, & De Troyer, (2001) further demonstrates a difference in the role of the external intercostal group as difference in rotation mechanics toward the top and back of the rib cage. This suggests the external intercostals located in these areas assist with inspiration and the external intercostals located towards the bottom and front of the rib cage in fact assist with expiration.

The levator costae are triangle shaped (Figure 1.8) and assist with inspiration via elevation of the ribs; the muscle fibres originate from the transverse process of the vertebra and insert on the caudal rib, however they are recruited secondary to the parasternal intercostal muscles (De Troyer et al., 2005).

Figure 1.8 The levator costae from a posterior view that identifies its triangular shape that assists with inspiration (adapted from Stone & Stone, 2011).

1.1.2.2 MUSCLES OF EXPIRATION

At rest, and during quiet breathing, expiration occurs passively via elastic recoil of the lungs and the chest wall, however during periods of intense exercise and with increasing intensity the muscles that make up the abdominal complex (Figure 1.9) are recruited to assist expiration (Sheel & Romer, 2012). Similarly to the accessory muscles of inspiration, this group has a dual purpose as they assist with movement, transfer of power, and forces between upper and lower extremities as well as stabilisation of the trunk and spine during human movement (Boussana et al., 2003; Heller, Challis, & Sharkey, 2009; Janssens et al., 2013). Recruitment of this group increases linearly with increased ventilatory demand and has a specific recruitment pattern that starts towards the end of the inspiration; activation begins deep with the transverse abdominus during moderate ventilatory demand and moving superficially to the external obliques and rectus abdominus during peak ventilation (Aleksandrova & Breslav, 2009).

Figure 1.9 The abdominal muscles that comprise the abdominal wall and assist with expiration during increased ventilatory demand (adapted from Stone & Stone, 2011).

1.1.2.2.1 ACCESSORY MUSCLES OF EXPIRATION

The internal intercostal and triangularis sterni muscles are primary accessory muscles recruited to assist the abdominal muscles with expiration (Aliverti, 2008a). The internal intercostal muscles are located in the ventral intercostal spaces (Ellis, 2008b) and positioned deep to the previously discussed external intercostal group. The recruitment is preferential from the bottom to the top of the chest wall (De Troyer et al., 2005) and is dependent upon ventilatory demand. The triangularis sterni lies deep to the parasternal intercostals and the sternum. They become activated in combination with the abdominal muscles below functional residual capacity (FRC); this encourages a passive rise of the chest wall during succeeding inspiration (De Troyer & Estenne, 1988). When under contraction, both groups reduce total lung capacity (TLC) which subsequently increases pleural pressure (*P*pl), assisting with deflating the ribcage by forcing air out of the lungs (Ratnovsky et al., 2008).

1.1.2.3 RESPIRATORY MUSCLE MORPHOLOGY

Respiratory muscles are morphologically and functionally skeletal muscles that differ only via a unique neural control that permit the musculature to contract rhythmically and repeatedly (Butler, 2007). When under contraction, they alter the dimensions of the thorax causing intra-thoracic pressure swings permitting inspiration and expiration (Miller, Beck, Joyner, Brice, & Johnson, 2002). Few studies have specifically analysed the morphology and muscle fibre type composition of the human respiratory muscles. Human cadaver studies have quantified that the internal and external intercostal muscles consist of ~60% type I muscle fibres which is slightly lower (~49%) in the diaphragm (Mizuno & Secher, 1989). This is a similar finding to those observed in the living human diaphragm (Nguyen et al., 2000), who quantified that Type II_A composition was similar between the internal intercostals and

diaphragm (~30%) but slightly lower (~17%) in the external intercostals. In contrast, Type II_B fibres was similar between the external intercostals and diaphragm (~25%) and only 1% in the internal intercostals (Figure 1.10). It was concluded here that the diaphragm had similar fibre characteristics to the vastus lateralis and the gastrocnemius. Indeed, it appears that the superior oxidative capacity of the diaphragm is not explained by muscle fibre composition but indeed by its vastly differing enzyme activity, vascular supply and mitochondrial density. These additional morphological adaptations however are not present in the accessory respiratory muscles, possibly due to the dual roles that these muscles play in facilitating movement, stability as well as thoracic excursion (Hodges et al., 2001; Ratnovsky et al., 2008).

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See the following reference for full image:

Mizuno, M., & Secher, N. H. (1989). Histochemical characteristics of human expiratory and inspiratory intercostal muscles. Journal of Applied Physiology, 67(2), 592–598.

Figure 1.10 Relative distribution of human slow twitch (type I), fast twitch A (type II_A) and fast twitch B (type II_B) muscle fibres for the expiratory and inspiratory intercostals, the costal diaphragm and the vastus lateralis. (adapted from Mizuno & Secher, 1989).

1.1.2.4 RESPIRATORY MUSCLE VASCULATURE

It is well established that the diaphragm and, albeit to a lesser extent the muscles that comprise the abdominal wall possess unique characteristics that make them less susceptible to fatigue (Sheel & Romer, 2012). As outlined above, this is not the result of differing morphological characteristics but could be the result of numerous vascular sources (Dempsey et al., 2006) and a unique resistance to vasoconstrictor influences (Aaker & Laughlin, 2002). Locomotor muscles are typically serviced by a single artery which provides oxygenated blood; the diaphragm however is supplied by multiple sources, and these include the superior and inferior phrenic arteries, intercostal artery and internal mammary artery, all of which originate from the thoracic branch of the descending aorta and serves to protect the diaphragm from ischemia (Comtois, Gorczyca, & Grassino, 1987). The vastus lateralis and diaphragm have similar muscle fibre characteristics, but there is a greater ratio of capillaries to muscle fibres within the diaphragm, which is aided by a reduced circumference of diaphragm muscle fibres thus reducing the diffusion distance (Mizuno & Secher, 1989). These mechanisms serve to increase the tolerance to increased respiratory demand during exercise. The complexity and vast vascular network that serve the respiratory musculature pose difficulty when attempting to monitor respiratory muscle blood flow, thus only few studies have directly attempted to monitor this in humans and the current understanding comes here from animal studies (Sheel & Romer, 2012). A series of studies from Manohar (1986a, 1986b, 1988) demonstrate in exercising ponies that the respiratory muscles receive 15% of total cardiac output ($\dot{Q}$) with 8% being delivered to the inspiratory muscles and 6% to the expiratory muscles during maximal exercise. Blood flow to the costal region of the diaphragm also increases 22 fold (12 mL·min·100g tissue to 263 mL·min·100g tissue, $P<0.05$) during exercise (Manohar, 1988) also demonstrating that intercostal perfusion was also elevated however to a lesser extent (~50%) than the diaphragm (Manohar, 1986a). It has also been estimated using

thermodilution in exercising humans that the respiratory system commands ~14 – 16% of $\dot{Q}$, to meet increased metabolic demand during strenuous cycling exercise (Harms et al., 1997a). Here, locomotor muscle blood flow was monitored during maximal cycling exercise under a control condition and when the work of breathing (WOB) was unloaded by ~50% using a proportional assist ventilator (PAV). During this trial, limb blood flow (20.3 ± 0.5 $L \cdot min^{-1}$) was unchanged when the respiratory muscles were unloaded.

1.1.3 THE WORK OF BREATHING

The WOB reflects the metabolic and/or energetic cost of the respiratory muscles to pulmonary ventilation and can be divided into two main categories: the elastic and resistive work (Butcher, Jones, Eves, & Petersen, 2006). The elastic WOB of breathing refers to the energy required to alter the shape of the anatomical structures involved with breathing and the resistive work of breathing is the energy required to overcome the resistance to airflow in the airways (Dominelli & Sheel, 2012). During exercise, the respiratory muscles must perform both elastic and resistive work. Elastic work is performed to expand the lung and chest wall and overcome the elastic recoil forces where resistive work increases when the air entering the airways becomes more turbulent during high airflow rates (Sheel & Romer, 2012). The principle of 'minimal effort' is thought to govern the WOB during exercise. This principle has been supported by experimental data, with mathematical modelling, that demonstrates the naturally occurring ventilatory response to exercise is associated with the minimal energy requirement from the respiratory musculature (Mead, Milic-Emili, & Turner, 1963). Furthermore, during exercise, ventilation occurs within a range of lung volumes that give the greatest total lung compliance (the change in pressure required to get a change in volume; ΔPressure/ΔVolume). The combination of the increase in tidal volume (V_T) and breathing frequency (f_B) occurs at a level that minimises dead space ventilation and lastly the respiratory

muscles shorten at an optimal length to maximise force production (Aliverti, 2008a; Dominelli & Sheel, 2012).

1.1.4 PHYSIOLOGY OF BREATHING AT REST AND DURING EXERCISE

The mechanical aspects of breathing can be described by the interaction of the lung, chest wall and includes the respiratory muscles that act upon them (Dominelli & Sheel, 2012), providing a pump like mechanism. At rest, pressure swings occur predominantly through autonomous contractions of the diaphragm that contract, descending downwards towards the abdominal cavity subsequently increasing volume and generating a negative pressure in the thoracic cavity (West, 2000). The negative pressure generated is proportional to the contraction of the diaphragm and is sub-atmospheric, prompting inspiration and air flow into the lungs (Aliverti, 2008a). Expiration at rest is a passive process where the energy used to provide thoracic excursion is released upon the cessation of inspiration allowing the respiratory system to reach functional residual capacity (FRC) via elastic recoil (Dominelli & Sheel, 2012). This is prompted primarily as the diaphragm and other obligatory inspiratory muscles relax and return to their neutral anatomical position, as this occurs thoracic volume reduces relative to inspiration and intra-thoracic pressure increases pushing air out of the lungs, prompting expiration (McConnell, 2013).

At the onset of exercise the demand for alveolar ventilation (V_A) is increased relative to both O_2 demand and CO_2 production to maintain acid-base balance close to homeostatic levels; this process is met with remarkable precision that matches metabolic demand and with minimal work (Sheel & Romer, 2012). Specifically here arterial O_2 levels must remain stable despite increased demand and a reduction in venous O_2 tension whilst simultaneous increases in CO_2 production must be expelled (Dominelli & Sheel, 2012). Changes within breathing mechanics occur autonomously with the onset of exercise via neural changes within the

cortical and brainstem centres that actively increase the recruitment and drive to both the obligatory and accessory respiratory musculature that act upon the ribcage to provide thoracic excursion and subsequent pressure swings (Aliverti, 2008a). Ensuring that V_A occurs without excessive demand is essential to maintaining metabolic efficiency during exercise where breathing pattern is altered across a range of ventilations. Changes in breathing mechanics that serve to meet increased ventilatory demand occur through changes in minute ventilation ($\dot{V}_E$), a product of both V_T and f_B. As shown in Figure 1.11, $\dot{V}_E$ progressively increases with increasing exercise intensity (Sheel & Romer, 2012; West, 2000). During mild exercise the predominant change occurs within V_T which expands into both inspiratory and expiratory respiratory volumes, achieved via increased inspiratory muscle recruitment and moderate increases in f_B. Changes in V_T plateau around ~60% of vital capacity (VC) as the elastic WOB becomes too high at this point meaning that subsequent increases in $\dot{V}_E$ are achieved through increases in f_B as breathing becomes tachypneic (Sheel & Romer, 2012). Increased f_B is achieved through reductions in total breath time (T_{TOT}), which relative to inspiratory time (T_I) occurs predominantly through reductions in expiratory time (T_E) as to protect V_T, thus increasing inspiratory duty cycle (T_I/T_{TOT}) by as much as 15% during maximal exercise (Dominelli & Sheel, 2012; West, 2000). Changes in V_T are achieved by the utilisation of both end-inspiratory lung volume (EILV), which occurs entirely due to rib cage expansion and also end-expiratory lung volume (EELV). The latter falls below FRC and occurs during light exercise as a result of expiratory muscle contraction thus reducing thoracic volume, utilising a greater proportion of TLC and reducing ventilatory dead space (Sheel & Romer, 2012).

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See the following reference for full image:

Sheel, A. W., & Romer, L. M. (2012). Ventilation and Respiratory Mechanics. Comprehensive Physiology. 2:1093-1142.

Figure 1.11 Schematic examples of the ventilatory responses to progressive increases in cycling work rate in a healthy young subject. Note the initial rise in V_T, which then plateaus. After this, further increases in ventilation are accomplished by increases in breathing frequency (Edited from Sheel and Romer, 2012).

Changes in ventilatory parameters must also operate with a high level of efficiency via numerous mechanisms. To maintain a minimal energetic cost across a range of ventilations as detailed above changes in f_B and V_T occurs in proportion to the required $\dot{V}_E$. The total work done by f_B is shown in Figure 1.12 where the lowest point on the curve represents the most efficient f_B for the different components of WOB during rest and exercise. Increases in V_T to 50% to 60% of VC during heavy exercise and subsequent reductions in EELV minimise the

elastic WOB of the inspiratory muscles, and allow the diaphragm to contract at the optimum length on its length tension curve (Dominelli & Sheel, 2012). Reduced EELV below FRC also allows for the storage of elastic energy in the chest and abdominal walls during expiration, which is used to produce a significant portion of the work required during inspiration, therefore reducing the WOB for the inspiratory muscles (Dominelli & Sheel, 2012; Sheel & Romer, 2012) and during heavy exercise accounts for ~33% of V_T (Aliverti, 2008a).

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See the following reference for full image:

Dominelli, P. B., Sheel, A. W., & Foster, G. E. (2012). Effect of carrying a weighted backpack on lung mechanics during treadmill walking in healthy men. European Journal of Applied Physiology, 112(6), 2001–2012.

Figure 1.12 Ideal breathing frequencies to minimise the work of breathing. Panel A represents a resting condition. Panel B represents an exercising condition. Adapted from Dominelli and Sheel., (2012).

1.2 EXERCISE INDUCED RESPIRATORY MUSCLE FATIGUE

During intense-exercise in healthy adults, the respiratory muscles can demand up to 10% of the total $\dot{Q}$ and up to 15% of oxygen uptake ($\dot{V}O_2$) in well trained athletes (Harms, Wetter, St Croix, Pegelow, & Dempsey, 2000). Respiratory muscle fatigue has been observed following high intensity exercise, and also following sustained periods of increased ventilatory demand and respiratory muscle recruitment. Fatigue is a multifaceted term that can be broadly described as a “loss in the capacity of a muscle developing force and/or

velocity, resulting from muscle activity under load, which is reversible by rest" (NHLBI, 1990, p. 474). Relative to the respiratory musculature, fatigue is evident from a reduced force output relative to baseline values (Romer & Polkey, 2008). Prior to the early 1990's it was suggested that the respiratory system, more specifically the lungs were not a limiting factor and indeed were overbuilt for exercise performance in untrained populations (Dempsey, 1986). The physical training adaptations however that are promoted amongst peripheral systems with regular exercise do not extend to adaptions within the pulmonary system, therefore exposing the respiratory system as a limiting factor for exercise performance in well trained individuals. Although it has been demonstrated that ventilatory parameters are not compromised and are indeed sustained with remarkable precision during exercise, high ventilatory demands pose specific challenges to the supporting musculature that power movement of the thorax and permit flow into the lungs. The first study to demonstrate this was observed by B. Johnson, Babcock, Suman, & Dempsey, (1993) whose findings are discussed in greater detail in Section 1.2.3, and has since stimulated a plethora of research attempting to understand the causal mechanisms and global effects to whole body performance that coincide with respiratory muscle fatigue. As detailed previously, the ventilatory response to exercise is met with near perfect precision that changes both f_B and V_T ($\dot{V}_E$). Consequently, increased recruitment of both obligatory and accessory muscles of inspiration occurs to regulate V_A with the metabolic demand imposed by exercise (Dempsey et al., 2006). The morphological and structural characteristics of the respiratory muscles, as detailed in Section 1.1.2.3, predisposes this group to fatigue during exercise that is high intensity (>85% $\dot{V}O_2$ peak), short duration (~15 min) and utilising large muscle groups (Romer & Polkey, 2008). More recently this has also been evident during prolonged submaximal exercise (Ross, Middleton, Shave, George, & McConnell, 2008).

The orientation of respiratory muscle fatigue can originate centrally or peripherally. Central fatigue is a reduction in voluntary force resulting from changes in the motor output from the central nervous system. The use of inspiratory resistive loads demonstrates central fatigue of the inspiratory muscles using bi-lateral phrenic nerve stimulation techniques (BPNS; described in Section 1.2.2) and twitch interpolation techniques that are superimposed upon a maximum inspiratory effort, demonstrating reductions in voluntary activation (Bellemare & Bigland-Ritchie, 1987). Several other techniques exist to measure the delivery of a motor-evoked potential by the use of transcranial electrical or magnetic stimulation of the motor cortex (Sheel & Romer, 2012); reductions in the amplitude of motor evoked potentials from transcranial stimulation indicates reduced excitability of the motor cortex due to supraspinal mechanisms. Transcranial stimulation has been used to show central diaphragmatic fatigue after whole-body exercise (Dayer et al., 2007; Verin et al., 2004) however, the consequences of a decrease in the motor-evoked potential remain unknown. Peripheral fatigue is a reduction in force output as a result of changes at or distal to the neuromuscular junction and can be subdivided into high-frequency and low-frequency fatigue (Sheel & Romer, 2012). High-frequency fatigue results in depression of the forces generated by a muscle in response to high-frequency stimulation (typically 50-100 Hz) and indicates altered neuromuscular junction transmission and/or reduced action potential propagation (Jones, 1996). Low frequency fatigue represents a reduction in force generation in response to low-frequency stimuli (e.g., 1-30 Hz, Edwards, Hill, Jones, & Merton, 1977) and represents impaired muscle excitation-contraction coupling (Allen, Lamb, & Westerblad, 2008). Both high and low-frequency fatigue has been reported for the diaphragm after periods of IRL and volitional hyperpnoea illustrating that high frequency fatigue recovers quickly, within about 10 to 20 min, whereas low-frequency fatigue may require hours to recover completely (Sheel & Romer, 2012).

Although the definition proposed earlier within this section adequately outlines the premise that underpins fatigue of the respiratory muscles collectively, it is however to distinguish between central and peripheral processes and also volitional and non-volitional techniques to assess exercise induced respiratory muscle fatigue.

1.2.1 RESPIRATORY MUSCLE FATIGUE: VOLITIONAL MEASURES

A common method used in clinical and exercise settings by respiratory physiologists to assess global respiratory muscle fatigue is the use of a handheld pressure meter as depicted in Section 2.3 to determine maximal inspiratory (P_{Imax}) and expiratory (P_{Emax}) pressures. These manoeuvres are non-invasive and characterised by quasi-static efforts which reflect the global pressure generating capacity of the respiratory muscles contracting in synergy (Martínez-Llorens et al., 2011); acting as a sufficient surrogate measures for the use of non-volitional and sniff pressure measures (Maillard, Burdet, Van Melle, & Fitting, 1998). Respiratory muscle fatigue using these measures is defined as a transient decrease in pressure after exercise (Sheel & Romer, 2012). This method has been used repeatedly throughout literature to determine the reductions in respiratory muscle strength. The use of acute inspiratory loading tasks by McConnell and Lomax (2006) demonstrate reduced P_{Imax} of 29.7% after participants inspired against a calibrated flow resistor with the nose remaining sealed at a set resistance of 60% P_{Imax} at 15 breathes·min^{-1}. Derchak, Sheel, Morgan, & Dempsey (2002) used expiratory resistive loads on both short (P_{Emax} 60%, DC_{exp} 0.4, f_B 30 breaths·min^{-1}) and long expiration breathing tasks (P_{Emax} 60%, DC_{exp} 0.7, f_B 15 breaths·min^{-1}) until task failure with no exercise. Mean exercise duration was 7 ± 3 min and both short and long duration expiration tasks resulted in expiratory muscle fatigue (16% and 14% respectively, $P<0.05$), with no reduction in control conditions. These findings were extended with the addition of whole body exhausting exercise, where 12 males completed 12 min runs

and observed reductions (~20%) in P_{Emax} to coincide with reductions in total distance covered, running speed and increased perceptual responses (Verges, Sager, Erni, & Spengler, 2007). Reductions in both P_{Imax} and P_{Emax} have been observed following whole body exercise over a broad range of exercise intensities, durations and modalities. Volianitis et al., (2001) observed reduced P_{Imax} of 11% in rowers completing a six minute maximal performance test which was furthered by Griffiths and McConnell (2007) who observed an 8% reduction (pre exercise P_{Imax} 129 ± 17 cmH_2O vs post exercise P_{Imax} 120 ± 22 cmH_2O, $P<0.05$), although no reductions in P_{Emax} were reported and reductions of 29% have been observed following a 200m swim (Lomax & McConnell, 2003). Reductions in P_{Imax} of between 12 and 18% are also reported after 20, 25 and 40 km cycling time trials (Johnson, Sharpe, & Brown, 2007; Romer, McConnell, & Jones, 2002c). Respiratory muscle fatigue has also been observed following continuous running activities such as marathon running (18% reduction, pre exercise P_{Imax} 118 ± 20 cmH_2O vs post exercise P_{Imax} 100 ± 22 cmH_2O, $P<0.05$, (Ross et al., 2008) and a 60 min treadmill trial, where 30 min was fixed at 60% $\dot{V}O_2$ peak and 30 min of free running (pre exercise P_{Imax} 156 ± 15 cmH_2O vs post exercise P_{Imax} 138 ± 17 cmH_2O, $P<0.05$ equivalent to a 12% reduction, (Tong, McConnell, et al., 2014). These findings clearly show that volitional measures of inspiratory and less frequently, expiratory muscle strength, are reduced following intense and prolonged bouts of exercise.

Although this technique maintains a degree of popularity in the assessment of maximal pressure generation in clinical and exercise settings, the validity of these measures have been frequently questioned due to an undesirable level of participant motivation and task learning involved with such measures. These techniques have been criticised for a lack of specificity as they target the entire recruitment process of the obligatory and accessory muscles which represents respiratory muscle fatigue as a global entity, failing to distinguish between fatigue of individual respiratory muscles (American Thoracic Society & European

Respiratory Society, 2002). Another potential limitation is that by using these measures it is not possible to identify whether post exercise reductions are the result of central or peripheral mechanisms (Sheel & Romer, 2012). However this method remains popular due to the simplicity in administering tests and with no need for specialist technical equipment and when sufficient time is given to familiarise participants these measures are highly reproducible (Hart et al., 2001; Romer & McConnell, 2004b).

1.2.2 RESPIRATORY MUSCLE FATIGUE: NON-VOLITIONAL MEASURES

Non-volitional methods for assessing respiratory muscle fatigue are used to assess the function of the respiratory muscles by measuring the diaphragmatic response to phrenic nerve stimulation (Hovey & Jalinous, 2006). This technique was first described in 1872 as a way of emulating natural respiration however pioneering work in this area was not conducted until the 1960's (Khong, Lazzaro, & Mobbs, 2010). This technique involves bilateral phrenic nerve stimulation to quantify diaphragmatic pressure prior to and following whole-body exercise (B. Johnson et al., 1993). The phrenic nerves lay most superficially at the spinous process at the 7th cervical vertebrae and pass down via the lungs and terminate at the diaphragm (Ellis, 2008a). Bilateral stimulation using magnetic stimulation techniques simultaneously stimulate all of the nerves that innervate the diaphragm, thus generating a pressure difference within gastric (P_{gas}) and oesophageal (P_{oes}) regions. These differences are detected by balloon catheters inserted via the nasal passage directly into this region, providing an indication of maximal force capability of the diaphragm ($P_{di} = P_{gas} - P_{oes}$). It is understood that P_{di} plateaus at around 40% of an exercise trial; whereas P_{oe} continues to increase, which is representative of a time dependant increase in accessory muscle recruitment to facilitate increased $\dot{V}_E$ and V_A. Despite post exercise reductions in P_{di}, the authors do not report impeded respiratory

function and it is widely accepted that the onset of diaphragm and accessory respiratory muscle fatigue does not result in reduced V_A.

Bi-lateral phrenic nerve stimulation techniques have been used to quantify diaphragmatic (Mills et al., 2014; Romer & Polkey, 2008) and gastric pressure (Taylor, How, & Romer, 2006; Taylor & Romer, 2008, 2009) prior to and following whole-body exercise. Johnson, Babcock, and Dempsey (1993) first used this technique to assess the extent to which the diaphragm became fatigued as a result of cycling at 85% $\dot{V}O_2$ peak (pre 27.5 ± 2.0 cmH_2O, post 22.6 ± 2.8 cmH_2O, $P<0.05$) and 95% $\dot{V}O_2$ peak (pre 24.9 ± 1.8 cmH_2O, post 21.5 ± 2.0 cmH_2O, $P<0.05$); importantly here, there were no observed reductions in P_{Imax} using volitional measures. The results of Johnson and colleagues (1993) were followed almost instantly by Mador et al., (1993) who observed similar reductions in P_{di} following short duration cycling (8.2 ± 4.1 min) in healthy untrained subjects ($\dot{V}O_2$ peak 35.6 $ml \cdot kg \cdot min^{-1}$). P_{di} reduced to 23.9 ± 3.7 cmH_2O from baseline (28.9 ± 3.7 cmH_2O, ~17%; $P<0.05$) following cycling exercise at 80% Wmax to volitional exhaustion, results which are comparable to Johnson et al., (1993). These studies were the first of their kind and provided initial insights into the fatigability of the diaphragm in response to exercise in trained and untrained individuals. Furthermore these findings have stimulated a plethora of research attempting to quantify and understand the mechanisms and consequences of exercise induced diaphragmatic fatigue.

These findings have since been furthered by Babcock, Pegelow, Taha, & Dempsey (1998) who observed reduced P_{di} (23.4 ± 3.3%) in response to twitch and 10 Hz tetanic BPNS techniques that were measured prior to, and following 9.9 ± 0.5 min maximal exercise at 95% $\dot{V}O_2$ peak. The findings demonstrate both low and high frequency fatigue of the diaphragm after intense, whole body endurance exercise, which by this group was later attenuated using PAV, detailed in the following section. More recently, Verges, Notter, Spengler, (2006)

observed diaphragm fatigue during constant-load high intensity endurance exercise at 85% peak power output (pre 44.2 ± 14.2 cmH_2O, post 24.5 ± 11.6 cmH_2O, $P<0.05$). The findings here have been extended to the expiratory muscles where magnetic stimulation of the spinal nerve roots has demonstrated a ~25% reduction in P_{ga} following cycling exercise at 90% $\dot{V}O_2$ peak until volitional exhaustion. This is exacerbated when preceded with an expiratory resistive load that serves as a pre-fatiguing breathing task (P_{Emax} 40%, DC_{exp} 0.7, f_B 15 breaths·min^{-1}); ultimately reducing exercise time by 33 ± 10% and inducing expiratory muscle fatigue (Taylor & Romer, 2008). This finding was later developed to demonstrate inspiratory (9-15%) as well as expiratory muscle fatigue (15-22%) when using expiratory resistive loads (Taylor & Romer, 2009).

The use of BPNS techniques is a common method used to objectively determine both intra-thoracic and gastric pressures. The technique is non-volitional, meaning it is independent of subject motivation enabling a muscles responsiveness to stimulation to be studied in isolation of central influences (Sheel & Romer, 2012). Non-volitional measures maintain a high degree of repeatability and reliability (within-day reproducibility ~5 to 10%) and validity (Hamnegard et al., 1995). However, the use of such techniques within clinical and exercise settings have been questioned due to the invasive nature that is required to insert the catheters which can be time consuming, costly, and cause a degree of discomfort for the participant.

1.2.3 FACTORS CONTRIBUTING TO EXERCISE-INDUCED RESPIRATORY MUSCLE FATIGUE

The current understanding suggests a number of contributing factors are important in the development of diaphragm fatigue which includes repeated, high level force output from the diaphragm and accessory respiratory muscles during high intensity exercise tasks. This notion is supported by significant relationships between diaphragmatic muscle pressure

production during the exercise and the degree of diaphragmatic fatigue observed after the exercise (Sheel & Romer, 2012). Greater direct evidence can be sought from studies that have sought to either unload or load the work of breathing during exercise tasks, thus attenuating or promoting fatigue of the locomotor musculature, respiratory musculature and affecting exercise performance (Babcock, Pegelow, Harms, & Dempsey, 2002; Romer, Lovering, Haverkamp, Pegelow, & Dempsey, 2006a). Babcock et al., (2002) measured P_{di} using BPNS at rest and following maximal cycling exercise at 85% $\dot{V}O_2$ peak, following a control trial performed for 9.6 ± 0.6 min, P_{di} was reduced 20–30% for up to 60 min after exercise. In a separate trial the WOB was unloaded by 40-50% using a PAV and participants duplicated the exercise time conducted for the control trial. Following this reductions in P_{di} were attenuated and $\dot{V}O_2$ was also 10-15% lower in the trial that incorporated PAV (Babcock et al., 2002). Prior to this a study from the same group determined that exercise induced diaphragm fatigue does not occur at reduced exercise intensities (<75% $\dot{V}O_2$ peak) performed both with and without PAV (Wetter, Harms, Nelson, Pegelow, & Dempsey, 1999).

It is acknowledged that the increased respiratory muscle output associated with increased exercise intensities itself cannot be solely responsible for the onset of exercise induced respiratory muscle fatigue. Following cycling exercise (13.2 ± 2.0 min; 86-93% $\dot{V}O_2$ peak), P_{di} was significantly reduced (26 ± 2.9%, $P<0.05$) in response to BPNS. No reduction in P_{di} was observed at rest when mimicking the same magnitude, frequency and duration of exercise to the limit of volitional tolerance (Babcock, Pegelow, McClaran, Suman, & Dempsey, 1995). In fact to induce identical levels of diaphragm fatigue, voluntary increases in P_{di} had to be elevated 1.5 - 2 times encountered during exercise (Babcock et al., 1995). It has since been postulated that in the absence of exercise the increase in ventilation is met with adequate blood flow, however during exercise the competition between the respiratory and locomotor musculature is increased (Harms et al., 1997a; Sheel & Romer, 2012). The

competition between diaphragmatic force output and O_2 transport to the diaphragm has been consistently demonstrated to be sufficient when exercise intensity is <80% $\dot{V}O_2$ peak (Sheel & Romer, 2012).

1.2.4 CONSEQUENCES FOR EXERCISE PERFORMANCE

Several approaches have been used to determine whether respiratory muscle fatigue affects exercise tolerance. One approach is to pre-fatigue the respiratory muscles at rest using acute inspiratory loads (IRL) and to observe whether subsequent whole body exercise tolerance is impaired (Romer et al., 2006; Romer & Polkey, 2008). These studies have shown a significant decrease in performance during subsequent heavy exercise (Mador & Acevedo, 1991; Verges, Sager, et al., 2007). The designs of such studies have been criticised for various reasons, including failure to objectively assess respiratory muscle fatigue. Some studies may have failed to induce significant respiratory muscle fatigue or indeed overestimate the normally occurring level of fatigue in response to whole body exercise prior to exercise tasks, resulting in questionable interpretation of the findings (Romer & Polkey, 2008). Additional limitations of these study designs are centred on the fact that blinding is not always possible hence, difficulty surrounding participant expectation to changes in exercise tolerance. There are also changes in breathing parameters, presenting difficulty in distinguishing whether the observed fatigue is solely caused by performance or indeed a combination of these factors. Other experimental approaches to determine the effect that respiratory muscle fatigue has on exercise performance partially unload the inspiratory muscles using a PAV (Harms et al., 2000; Romer et al., 2006). These approaches partially unload the inspiratory muscles and have demonstrated increased time to exhaustion, and the rates of rise of $\dot{V}O_2$ and perceptual responses specific to respiratory and limb discomfort were reduced during constant-load exercise at increased work rates (<90% $\dot{V}O_2$ peak) but not at lower work rates (Harms et al.,

2000; Romer et al., 2006). Although unloading studies positively affect performance outcomes, an imposed limitation is that it is difficult to determine whether the positive effect of reducing respiratory muscle work on exercise tolerance is attributable to the relief of respiratory muscle fatigue, or whether there is a perceptual benefit obtained by relieving the discomfort attending high levels of respiratory muscle work.

The consequences of respiratory muscle fatigue, as outlined to date has negative implications for performance on whole body exercise tasks and the onset of such fatigue prompts a series of cardiorespiratory reflexes. These result in systemic neural and chemical alterations that modify the global physiological responses seeking to sustain sufficient P_{di} and V_A. The key mechanisms here are concerned with cardiorespiratory and perceptual responses.

1.2.5 PERCEPTUAL RESPONSES DURING EXERCISE

The onset of exercise is met with increased perceptual strain and is acknowledged as a key determinant in exercise capacity and performance (Amann, 2012; Dempsey, Blain, & Amann, 2014). These measures can be characterised as whole body exertion measured typically using the 6-20 Borg scale (Borg, 1982) or, as commonly practiced within literature these can be subdivided to specific regions of the body, namely to identify locomotor muscle and breathing discomfort (Verges, Boutellier, & Spengler, 2008). During intense exercise and more specifically in the presence of respiratory muscle fatigue, perceptual responses are exacerbated (Dempsey et al., 2006; Romer & Polkey, 2008). The specific mechanism behind increased limb and breathing discomfort is detailed in Section 1.2.6; the focus here is to outline the mechanisms and current theoretical understanding of increased perceptual responses during exercise and under conditions of respiratory muscle fatigue. Recent years has seen numerous centrally orientated mechanisms postulated within literature that seek to outline the causal relationship between exercise performance and increased perceptual strain,

recently reviewed by Dempsey et al, (2014). One suggestion is the anticipatory feedback model, which proposes that perceptual responses to exercise are forecast via a 'conscious template' that is derived from knowledge and experience of the required performance task. It is integrated with afferent feedback from peripheral sources (i.e. fatiguing contractions of the diaphragm and other respiratory musculature) to develop a 'perceptual template', which during exercise is continuously compared with the 'conscious template'(Tucker, 2009). Continuous adjustments to workload and perceptual responses are altered from peripheral input (afferent discharge) to ensure that the conscious effort is "acceptable" during exercise tasks and to avoid unfavourable disturbances from homeostasis within the peripheries (Tucker, 2009). Although plausible, the complex mechanism and interaction between central and peripheral processes involved in the regulation of perceptual responses are still poorly understood (Amann, 2012).

One key mechanism that has unprecedented importance in the modulation of dyspnoea and perception of effort is the importance of afferent feedback. These ascend to the central nervous system from their origin and are important in evoking the appropriate ventilatory and circulatory responses to exercise; which are critical pre-requisites for preventing premature fatigue and attaining optimal exercise performance (Amann, 2012; Butler & Gandevia, 2008; Dempsey et al., 2014). The neural feedback projects from the peripheral nervous system via skeletal muscle afferents which are richly innervated within skeletal and respiratory musculature. They project via the dorsal horn to the primary sensorimotor cortex located within the central nervous system, acting as a continuous feedback mechanism between the central and peripheral nervous systems (Dempsey et al., 2014). Type III (mechanoreceptors) and Type IV (metaboreceptors) muscle afferents are richly sourced within respiratory and skeletal musculature and studies have shown increased firing rate during dynamic exercise (Adreani, Hill, & Kaufman, 1997; Kaufman, Hayes, Adreani, & Pickar, 2002; Pickering &

Jones, 2002) and fatiguing contractions of the respiratory musculature (Amann & Dempsey, 2008). Increased afferent discharge is proportional to metabolite accumulation and total work done by the muscle group to preserve homeostasis and regulate central motor drive (Amann, 2012).

Clinical studies have used the neuromuscular blockades and anaesthetics to block the central projection of muscle afferents to the central nervous system during whole body endurance exercise. The results of these studies appear controversial with some finding attenuated, similar or even increased ventilatory responses during exercise (Freund, Rowell, Murphy, Hobbs, & Butler, 1979; Friedman et al., 1993; Kjaer et al., 1999; Smith et al., 2003). The inconsistency surrounding such data from clinical studies can be attributed to the differing blockades used during trials, the use of epidural anaesthetics necessitate careful interpretation due to the role these play in blocking both afferent and efferent activity (Amann, 2012). This promotes muscle weakening prompting an increase in central motor command to facilitate exercise performance and thus augmenting ventilatory (and cardiovascular) responses to exercise (Dempsey et al., 2014). Recent studies that were designed to skirt the confounding impact of epidural anaesthetics do however; provide valuable insights into the effects of muscle afferents on the circulatory and ventilatory response to whole body endurance exercise via the use of intrathecal fentanyl. Here, the central projection of group III/IV muscle afferents affects both central motor drive and muscle's force generating capacity (Amann et al., 2010; Amann, Runnels, et al., 2011; Dempsey et al., 2014). The outcome demonstrates that when group III/IV muscle afferents from the lower limbs are blocked during endurance exercise pulmonary ventilation and circulation are substantially compromised, causing ventilatory and metabolic acidosis which combine to accelerate the development of peripheral locomotor muscle fatigue during exercise (Amann, Blain, et al., 2011). This suggests that continuous sensory feedback from

skeletal muscles might depict a vital component in providing cardio respiratory responses to exercise and also a high capacity for rhythmic endurance exercise (Amann, 2012; Dempsey et al., 2014).

Recent evidence suggests that the orientation of afferent feedback and subsequent increases in perceptual responses that ensues is indeed independent of the initial discharge source. Grippo et al., (2010) observed elevated dyspnoea either side of a leg fatiguing protocol that sought to induce low-frequency fatigue of the m.quadriceps when compared with a control group who rested with quiet breathing. The fatigue protocol included sustaining MVC force >80% of peak power output until task failure, following 15 s rest contraction was resumed and this was repeated until participants could not sustain 50% peak power output for more than 5 s. Dyspnoea was elevated prior to the fatigue protocol via the use of a resistive breathing task which involved 5 min of resistive breathing against five differing resistive loads (10, 18, 30, 40 and 90 cmH_2O), which was exacerbated in the experimental group ($P<0.05$) and not in the control ($P>0.05$). Sharma, Morris, & Adams (2015) have furthered these findings by specifically isolating the dyspnoea response to fatiguing leg exercise. They observed increased dyspnoea and no subsequent change in ventilatory pattern following a leg fatiguing protocol. Participants completed knee extensor exercises with a resistance equal to 40% of participants' body mass until task failure, then following 20 s rest a second bout was conducted again until task failure. This was repeated a further four times with the rest between sets increasing by 10 s. This was sufficient in reducing MVC peak force by 22% and importantly increased perceptual discomfort by 29% ($P<0.05$). This evoked an increase in dyspnoea that is independent of changes in ventilatory pattern and is attributed to an increase in both mechanical and metabolic stress within the skeletal muscle resulting in increased group III and IV afferent discharge (Grippo et al., 2010). Afferent discharge from peripheral bases ascends to the sensorimotor cortex via the dorsal horn at which point the orientation of

afferent sources are not distinguishable, resulting in increased perceptual strain and is a key limiting factor for exercise performance (Amann, 2012; Harms et al., 2000; Romer & Polkey, 2008). A second and important reflex that occurs with the onset of respiratory muscle fatigue and a result of increased afferent discharge is the respiratory muscle metaboreflex.

1.2.6 CARDIO-RESPIRATORY INTERACTIONS DURING EXERCISE

Fatiguing contractions of both the diaphragm and the accessory musculature increases accumulating metabolites, subsequently stimulating increased discharge from group III and IV afferents. This projects to the CNS and triggers an increase in sympathetic efferent response that increases muscle sympathetic nerve activity (MSNA) and limb vascular resistance, autonomously redistributing $\dot{Q}$ in favour of the fatiguing respiratory musculature to sustain V_A (Dempsey et al., 2006; Derchak et al., 2002). This is a likely mechanism for a reduction in exercise performance as well as contributing to increased perceptual responses. The use of inspiratory and expiratory resistive loads (~60% P_{Imax}) induces fatigue of the diaphragm and abdominal muscles, leading to a time-dependant increase in muscle sympathetic nerve activity measured at the peroneal nerve (Derchak et al., 2002; St Croix, Morgan, Wetter, & Dempsey, 2000). In both inspiratory and expiratory muscles increased MSNA and subsequent increases in limb vascular resistance are attributed to an increase in the discharge of chemo-sensitive type III and IV afferent fibres located in the fatiguing diaphragm and expiratory muscles as a result of sustained increases in the WOB. Indeed type III and IV afferent fibres are highly sensitive to metabolite accumulation and provide compelling evidence in support of a metaboreflex; in particular lactate is known to significantly increase the discharge frequency of afferent fibres (Sheel et al., 2001; Sinoway, Hill, Pickar, & Kaufman, 1993). This is supported via the infusions of lactate into the phrenic or deep circumflex iliac arteries which significantly increased mean arterial blood pressure

(21 ± 7%), reduced both $\dot{Q}$ (6 ± 2%) and hind limb blood flow (20 ± 9%) at rest and during exercise in dogs. Simultaneously this reduced total systemic, hind limb perfusion and abdominal expiratory muscle vascular conductance (Rodman, Henderson, Smith, & Dempsey, 2003).

Increase discharge of these fibres prompts a sympathetically mediated efferent response which causes a time-dependent reduction in limb vascular conductance secondary to an increased limb vascular resistance (Sheel, 2002; Sheel & Romer, 2012). Although the increase in sympathetic efferent activity and norepinephrine spillover is not specific to any particular vascular bed, diaphragm perfusion is protected since the α1-adrenergic receptors of the diaphragm arterioles have reduced sensitivity to vasoconstrictive stimuli as the diameter of the blood vessels did not change with progressively larger doses of nor-adrenaline (Aaker & Laughlin, 2002), thus prompting the term respiratory muscle metaboreflex as summarised in Figure 1.13.

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See the following reference for full image:

Dempsey, J. A., Romer, L. M., Rodman, J., Miller, J., & Smith, C. (2006). Consequences of exercise-induced respiratory muscle work. Respiratory Physiology & Neurobiology, 151(2), 242–250.

Figure 1.13 Schematic of the origin and consequences of the respiratory muscle metaboreflex (Dempsey et al., 2006).

Reductions in limb vascular conductance and subsequent blood flow have also been observed following acute inspiratory loading. Harms et al., (1997) increased the work of breathing during maximal whole-body cycling exercise to the limit of volitional tolerance with the use of mesh screens, increasing the work of breathing by 5 $cmH_2O \cdot L \cdot_s^{-1}$. This reduced limb vascular conductance by ~0.9 $L \cdot min^{-1}$ (~33%) which was measured via thermodilution techniques. Although extraction of O_2 remained unchanged, when the inspiratory muscles were unloaded using a PAV (~50%), limb perfusion was sustained at near resting levels. A significant correlation was revealed between the WOB and limb blood flow (r = 0.84 to 0.9, P<0.05) and limb $\dot{V}O_2$ (r= 0.77, P<0.05). Significant correlations were also reported between increased limb vascular resistance and nor-adrenaline spill over in the limb; however these physiological responses were not observed during sub-maximal exercise at 50 and 75% $\dot{V}O_2$ peak (Wetter, Harms, Nelson, Pegelow, & Dempsey, 1999). This is suggestive of a unique interaction between the WOB and limb blood flow. The findings were supported in a similar study by Romer et al., (2006), and although no method was used to observe limb muscle perfusion, contractile function was assessed instead using electromyography and magnetic stimulation of the femoral triangle to stimulate the m.quadriceps. In this study endurance trained cyclists completed three identical cycling trials at >90% $\dot{V}O_2$ peak with additional restriction/reductions in WOB. This was repeated with an additional inspiratory load (3–7 $cmH_2O \cdot L \cdot_s^{-1}$) created similarly to Harms et al., (1997) by adding mesh screens to the inspiratory line, when the inspiratory muscles were unloaded using a PAV (~2.5 $cmH_2O \cdot L \cdot_s^{-1}$). The authors report a reduction in the exercise time of 39 ± 6 % during the loaded breathing trial compared with the trial when the work of breathing was unloaded (~56%) where it was observed that exercise induced quadricep fatigue was attenuated (~33%) against the control (Romer et al., 2006).

Reduced limb vascular conductance and subsequent redistribution of $\dot{Q}$ via vasoconstrictor influences within the peripheries diverts fresh oxygenated blood in favour of the respiratory musculature via increased sympathetic vasomotor outflow sources. This poses additional demands to locomotor muscle groups, which may increase both peripheral locomotor fatigue and perceptions of limb discomfort during high-intensity (>90% $\dot{V}O_2$ peak) exercise (Romer et al., 2006). When the inspiratory muscles were unloaded (37% lower vs CON) in a similar fashion as described by Harms et al., (1997), the time-pressure response was reduced and the level of fatigue observed within the quadriceps muscle was attenuated (~28% versus CON). This coincides with reductions in limb discomfort and effort perceptions when compared with control conditions of the same exercise duration (13.2 ± 0.9 min). Equally the use of an IRL increases force output of the inspiratory muscles (51% higher vs CON) and quadriceps fatigue was exacerbated (~20% versus CON) alongside reductions in exercise duration (7.0 ± 0.6 min, 39% lower than CON), and heightened limb discomfort. The reduced relative demand upon the respiratory muscles, is a common observation following acute and chronic loading strategies, and albeit to a lesser extent, may indeed be advantageous in sustaining or in fact increasing exercise performance.

1.3 Respiratory Muscle Loading

Loading of the respiratory muscles is concerned with creating a resistance to breathe and is common within research; two differing methodologies exist and includes acute and chronic respiratory loading. The use of chronic loading strategies are common within respiratory muscle training research via the use of progressive overloads which results in numerous beneficial adaptations and importantly results in increased exercise performance; this will be discussed in greater detail in Section 1.3.2. The use of acute loading is also popular and is used in numerous capacities to assess respiratory muscle performance.

1.3.1 ACUTE RESPIRATORY MUSCLE LOADING

Acute inspiratory loading has been used within research to test the endurance of the respiratory musculature (Brown, Johnson, & Sharpe, 2014; Verges, Lenherr, Haner, Schulz, & Spengler, 2007). In research, loaded incremental breathing tasks to the limit of tolerance were conducted until the point of task failure and have served as a useful tool to assess respiratory muscle endurance pre and post chronic loading (Leddy et al., 2007; Verges, Lenherr, et al., 2007). Acute inspiratory loading is also used during resistive breathing tasks that utilise high intensities (~80% P_{Imax}) to induce respiratory muscle fatigue and allow observation of the physiological consequences upon the WOB and also exercise performance (Dempsey et al., 2006; Guenette & Sheel, 2007; Mador & Acevedo, 1991; McConnell & Lomax, 2006; St Croix et al., 2000; Verges et al., 2006). Repeated efforts against an inspiratory and expiratory load provides an overload stimulus that prompts task failure and significant diaphragm fatigue and onset of the respiratory muscle metaboreflex (McConnell & Lomax, 2006; Romer & Polkey, 2008; Verges, Lenherr, et al., 2007; Verges et al., 2006).

Reducing the inspiratory load (~40% P_{Imax}) provides an effective warm-up stimulus that specifically primes the respiratory musculature for exercise. In the context of this thesis the use of acute inspiratory loading is used as potential warm up stimulus for the inspiratory musculature that are neglected by whole body warm-ups, which are an important feature of physical exercise preparations. Whole body warm ups constitute an improvement in performance by targeting large locomotor muscle masses and accelerate oxygen uptake ($\dot{V}O_2$) at the locomotor muscles through a variety of central and peripheral mechanisms (Bishop, 2003a, 2003b). The typical design of priming activities however poses little challenge to the ventilatory processes including the respiratory muscles (Johnson, Gregson, Mills, Gonzalez, & Sharpe, 2014; Tong & Fu, 2006; Volianitis, McConnell, Koutedakis, & Jones, 2001) which is unexpected since the respiratory muscles are imperative in sustaining V_A and regulating

perception of effort and central motor output (Dempsey, Sheel, St Croix, & Morgan, 2002; Marcora, 2009).

Previous research has demonstrated that adopting respiratory muscle warm ups increases the peripheral excitability and EMG activity of the diaphragm and intercostal muscles (Hawkes, Nowicky, & McConnell, 2007; Ross, Nowicky, & McConnell, 2007), causing transient increases in P_{Imax} (Johnson et al., 2014; Ross et al., 2007) and coincidently reducing observed rates of respiratory muscle fatigue (Volianitis, McConnell, Koutedakis, & Jones, 2001). Ultimately this leads to improved exercise performance through increased time to the limit of tolerance in intermittent running to exhaustion (Lin et al., 2007; Lomax, Grant, & Corbett, 2011; Tong & Fu, 2006) and distance covered in a 6 min all out rowing time trial (Volianitis, McConnell, Koutedakis, & Jones, 2001). Tong and Fu (2006), observed increased time to the limit of tolerance on an intermittent running performance (9.1%, $P<0.05$) when the authors combined acute inspiratory loading with an active warm up. Similar positive improvements were reported during badminton footwork performance (7.8%, $P<0.05$) from the same group (Lin et al., 2007). Lomax et al (2011) also observed a 5% increase in an intermittent run to exhaustion when adopting the protocol of Tong and Fu (2006). Each study described above incorporated a standardised active warm up comprising 5 min self-paced treadmill running, 10 min of static stretching, and finally 5 min of self-paced running on a track that was consistent across all trials. The IMWU was performed between static stretching and free running and was identical to the present methodology (Lin et al., 2007; Lomax et al., 2011; Tong & Fu, 2006).

In studies investigating cycling and rowing performance, the outcomes are varied. Distances covered and mean power on a 6 min all out rowing simulation test was improved (3.2%, $P<0.05$) when combining a specific rowing warm up with an inspiratory muscle warm up. The active warm up mimicked pre-competition exercise; 5 min of light jogging on a

treadmill, 10 min of unspecified stretching, 12 min of rowing with increasing intensity achieved via alterations in stroke rate, and concluded with 5 sprints at 30s, 45s and 60s at a stroke rate of 26-32 strokes·min^{-1}. Total distance covered and average power output were also increased and perceived dyspnoea and post exercise reductions in P_{Imax} compared with a sub-maximal and specific rowing warm-up per se. In comparison, Johnson et al (2014) observed similar increases in P_{Imax} to others (Lin et al., 2007; Lomax et al., 2011; Tong & Fu, 2006; Volianitis, McConnell, Koutedakis, & Jones, 2001) however the addition of this to a 15 min cycling warm-up consisting of 3 x 5 min segments at 70, 80 and 90% of the gas exchange threshold did not improve 10 km cycling time trial performance (P>0.05). Although there is a dearth of literature surrounding the key mechanisms that constitute improvements in exercise performance, the application of such techniques has observed contrasting findings. The application of specific respiratory warm ups via acute inspiratory muscle loading appears logical, and combining this with traditional whole body exercise warm ups could provide a quick and meaningful benefit to exercise performance however the research surrounding the potential benefits requires further investigation. Combining both acute and chronic inspiratory loading identified both techniques serves a very different purpose. Both provide a benefit to performance with the greatest benefit derived via chronic loading methods that promote morphological and structural adaptations (Lomax et al., 2011).

1.3.2 CHRONIC RESPIRATORY MUSCLE LOADING

The respiratory muscles are very plastic and adapt structurally to loading stimuli, this has been observed in labouring tasks (Rochester et al., 1982), weightlifting (DePalo, Parker, Al-Bilbeisi, & McCool, 2004) and powerlifting (Brown et al., 2013). Chronic loading techniques specifically target changes in respiratory muscle morphology and increased respiratory muscle function have been frequently used within literature, collectively termed

respiratory muscle training (RMT). Delhez, Bottin, Thonon, & Vierset, (1966) observed a 37 ± 23% increase in inspiratory muscle strength after completing 3 Muller manoeuvres daily over an 8 week period. Since this initial, and albeit accidental, discovery of RMT methods in a clinical setting, the knowledge and application of RMT has gained increasing popularity as it has been demonstrated to be an effective ergogenic aid for athletic performance (McConnell, 2009). This is due in part to increased understanding that the known training principles for peripheral muscle groups, is indeed applicable to the respiratory musculature. Leith and Bradley (1976) were the first to recognise RMT in a study that observed a 19% increase in the maximal sustainable ventilatory capacity and a second group observed a 55% increase in P_{Imax} following 5 weeks of voluntary hyperpnoea training (outlined in Section 1.3.2.2). It is important to note that at the time of the findings of Leith and Bradely (1976) the physiological consequences of the work of breathing and repercussions of respiratory muscle fatigue, as outlined in Section 1.2.3, were not known. Together with the knowledge and further understanding that respiratory musculature is a limiting factor to exercise and the implications for whole body performance, RMT has since received considerable attention from research groups to help understand how RMT can thus improve whole body performance (HajGhanbari et al., 2013; Illi, Held, Frank, & Spengler, 2012). The findings from literature regarding improved performance are however inconsistent and the mechanisms are not fully understood, but these will be reviewed in greater detail in Sections 1.3.6 respectively.

1.3.2.1 RESPIRATORY MUSCLE TRAINING TECHNIQUES

Techniques of RMT seek to target the force-velocity principle of training specificity established for peripheral skeletal muscles, which is applicable to the respiratory musculature (Romer & McConnell, 2003) due to the previously discussed morphological adaptations

described in Section 1.1.2.3. Acknowledging that inspiratory airflow is proportional to the velocity of muscle shortening and inspiratory pressure is proportional to force generation (Romer & McConnell, 2003), there are three main techniques that exist to train the respiratory musculature: voluntary iscocapnic hyperpnoea (VIH), flow resistive loading (FRL) and pressure-threshold loading (PTL). Increased maximal inspiratory flow occurs with high-velocity low-pressure training (such as VIH) and seeks to improve respiratory muscle endurance, whereas training with high-force and low-velocity contractions (as found in FRL) improves muscular strength (Romer & McConnell, 2003). It is possible through PTL training to combine high resistive loads and high flow rates during RMT; targeting improvements in both respiratory muscle strength and endurance (Illi et al., 2012; McConnell, 2009; Tzelepis, Kadas, & McCool, 1999) as shown in Figure 1.14. Each technique as discussed in the following sections has a differing focus (Cahalin & Arena, 2015), all of which are excellent training tools, promoting positive adaptions in respiratory muscle function and whole body exercise performance. Therefore a subsequent review is required to determine a suitable training device and exercise protocol to be used within this thesis.

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See the following reference for full image:

Romer, L. M., & McConnell, A. K. (2003). Specificity and reversibility of inspiratory muscle training. Medicine and Science in Sports and Exercise, 35(2), 237–244.

Figure 1.14 Maximal inspiratory pressures (P_I) and inspiratory flow rates ($\dot{V}_I$) pre and post 9 weeks of pressure threshold IMT (Adapted from Romer & McConnell, 2003).

1.3.2.2 VOLUNTARY ISOCAPNIC HYPERPNOEA (VIH)

As outlined previously VIH utilises high-velocity flow rates and low levels of pressure and targets increases in respiratory muscle endurance (Romer & McConnell, 2003). Adopting this technique requires participants to increase $\dot{V}_E$ to a prescribed level and sustained for a given period of time. Typically a training session is conducted at 50 to 85% of the individual's maximal voluntary ventilation (MVV) with a f_B typically of 30 to 45 breaths·min^{-1} and a V_T of 50-60% of VC (~2.5 to 3.0 L) for 30mins a day, 3-5 times per week for up to four weeks (Verges et al., 2008). During sessions there is a need to constantly monitor flow and breathing pattern to ensure the target intensity is met, which is pivotal to the success of VIH techniques. As a result, it is common that most devices are fitted with a screen, as shown in Figure 1.15, to allow training intensity to be regulated.

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www.spirotiger.com

Figure 1.15 Diagrammatic representation of a commercially available training device used during voluntary hyperpnoea training protocols (www.spirotiger.com; accessed 09.04.2015)

The device contains a CO_2 re-breathing bag that is necessary due to rapid reductions in the arterial partial pressure of CO_2 (P_aCO_2) occurring as a consequence of manually increasing $\dot{V}_E$ above resting levels. This causes light headedness, dizziness and fainting and as to avoid this additional CO_2 is added to each inspiratory effort. The bag inflates to 50% of VC during expiration and is comprised of atmospheric CO_2 and CO_2-enriched air from the re-breathing bag is inspired during subsequent inspiratory efforts, sustaining P_aCO_2 levels (Leddy et al., 2007). VIH has demonstrated substantial improvements in ventilatory endurance; Boutellier & Piwko (1992) observed increases in breathing endurance in well trained individuals following four weeks of VIH training, 6.1 ± 1.8 min to 40 min which was highlighted as a cut-off point. Comparable observations were noted in moderately trained healthy males (pre training: 24.7 ± 7.1 min, post training: 40.0 min) which was used as a cut off (Verges, Lenherr, et al., 2007) and in well trained competitive runners (pre training: 14.71 ± 6.68 mins, post training: 36.01 ± 10.33 mins; Leddy et al., 2007). The use of voluntary hyperpnoea elicits notable improvements in respiratory muscle endurance as it solely targets the flow axis of the

force-velocity relationship and also athletic performance in a recent review by (HajGhanbari et al., 2013). This method of training contains a high level of ecological validity as it offers the highest level of specificity but has been criticised; sessions are often time consuming, requires supervision and require high levels of participant motivation (McConnell, 2013; Romer & McConnell, 2004).

1.3.2.3 FLOW RESISTIVE LOADING (FRL)

Flow resistive loading specifically targets the inspiratory muscles through the use of high force and low velocity contractions as participants inspire through a variable sized aperture which provides the resistance during training. The specific effect of flow-resistive loading on the inspiratory muscle force-velocity curve is however dependent upon the level of inspiratory flow and the amount of resistance determined, according to Ohm's law, that considers how the velocity of air flow (current) is altered by resistance (V= I x R). Airway resistance is therefore a product of airway diameter and airflow, and determines airflow resistance during FRL RMT. During inspiration the pressure generated by the inspiratory muscles (i.e., training resistance) is dependent upon inspiratory flow rate. Consequently it is imperative to monitor breathing during training as a decrease in inspiratory flow results in a reduction in airway resistance and a further reduction of the inspiratory load (McConnell, 2013). Novel approaches to overcome the suggested limitations includes the use of incremental respiratory endurance (TIRE) systems (Cahalin & Arena, 2015). These sessions are characterised by a serial presentation of submaximal isokinetic-like profiles that utilise a percentage of P_{Imax} and a progressive work to rest ratio. Sessions entail 6 sets of sustained inspiratory efforts (max 36 repetitions) at 80% P_{Imax} that are separated by rest periods that reduce from 60 s, but sessions are terminated prematurely if the participant completes the prescribed repetitions or the generated pressure does not meet the pre-determined reference

value (Cahalin & Arena, 2015; McConnell, 2013). Training typically lasts from 4–10 weeks which contains 3 sessions per week (Enright, Unnithan, Heward, Withnall, & Davies, 2006; Gething, Passfield, & Davies, 2004) and requires specialist equipment and computer software to operate such training.

Flow resistive loading targets improvements in both respiratory muscle strength and endurance, P_{Imax} was increased by 18–41% after 4 to 6 weeks and improvements in inspiratory muscle endurance have also been observed with sustained inspiratory pressure improved from 36 to 47% (Enright et al., 2006; Gething et al., 2004). Diaphragm thickness via induced hypertrophy at functional residual capacity has been reported to increase by 12% (Enright et al. 2006); this adaptation is detailed further in Section 1.3.6. The ecological validity of such techniques described here is questioned as it does not appropriately represent dynamic inspiratory muscle function observed during whole-body exercise (McConnell, 2013). Although FRL targets changes in respiratory muscle strength and endurance, the reliance on equipment required to monitor breathing pattern limits the use of such techniques to a laboratory setting where specialist personnel and equipment are present (Cahalin & Arena, 2015).

1.3.2.4 PRESSURE-THRESHOLD LOADING (PTL)

Unlike VIH and FRL, which target a singular axis of the force-velocity relationship, PTL is near flow-independent (Caine & McConnell, 2000). As a technique it targets both axes and seeks to increase both inspiratory muscle strength and endurance, and is commonly referred to within literature as IMT. Romer and McConnell (2003) demonstrated the specificity of PTL as a IMT technique in a study where participants were assigned to one of four groups and undertook pressure-threshold IMT with the following loads; high resistance-low flow (A), low resistance high flow (B), intermediate resistance and flow (C) or no

pressure-threshold IMT (D). As demonstrated in Figure 1.16, Group A exhibited the largest increase in inspiratory pressure, Group B a large increase in inspiratory flow rate, Group C uniform increases in inspiratory pressure and flow rate and Group D no changes in inspiratory pressure or flow rate. Pressure-threshold training requires the participant to produce a negative pressure that is sufficient to overcome a pre-set threshold load and permit inspiration through the opening of a spring loaded solenoid valve as shown in Figure 1.17. Once the pressure cannot be sustained, the valve closes and passive expiration ensues. Each inspiratory effort is initiated from RV in an attempt to maximise V_T and because rapid inspiration is followed by a protracted expiration there is no apparent need for supplemental CO_2 or a re-breathing bag as changes in PCO_2 are minimal. This method is also near flow-independent, which means that during the active phase of inspiration the generation of pressure is independent of flow (Caine & McConnell, 2000), negating the need to monitor breathing pattern during sessions.

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Romer, L. M., & McConnell, A. K. (2003). Specificity and reversibility of inspiratory muscle training. Medicine and Science in Sports and Exercise, 35(2), 237–244.

Figure 1.16 Relative changes in maximum inspiratory flow rate (A) and maximum P_{Imax} (B) after 3, 6 and 9 weeks of PTL IMT with high resistance-low flow (Group A), low resistance-high flow (Group B), intermediate resistance and flow (Group C) or no pressure-threshold IMT (Group D). Significant difference from pre-intervention * $P<0.05$ from the preceding measurement point ^ $P<0.05$. Values are mean ± SE. Figure adapted from Romer and McConnell (2003).

A typical training session using this method consists of 30 breaths at 50% P_{Imax} twice daily for 4-6 weeks. Greater intensities have been used within literature but yields no greater

adaptation to respiratory muscle strength (McConnell, 2013; Romer & McConnell, 2003, 2004). Adaptions of strength and endurance within skeletal muscles occur following as little as 12 sessions (Staron et al., 1991) so it is important to sustain the training stimulus at 50% P_{Imax} to ensure constant overload of the muscle group and promote continued adaptation (HajGhanbari et al., 2013). This see's reassessment of P_{Imax} and calibration of the device on a bi-weekly basis to maintain the optimum training threshold, throughout the intervention participants can periodically increases the resistance via alterations to the valves opening pressure by tightening the tension screw cap to accommodate for rapid and temporal improvements in P_{Imax}.

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See the following reference for full image:

Caine, M. P., & McConnell, A. K. (2000). Development and evaluation of a pressure threshold inspiratory muscle trainer for use in the context of sports performance. Sports Engineering, 3(3), 149–160.

Figure 1.17 Example of a commercially available pressure threshold inspiratory muscle training device (accessed and edited from Caine & McConnell, 2000).

A recent review by HajGhanbari et al (2013) observed that the use of threshold IMT programmes such as PTL elicited the greatest improvements in P_{Imax} compared with alternative training methods. Typically research using PTL IMT has reported increases in

P_{Imax} between 17–55% following a 6 week intervention (Brown, Sharpe, & Johnson, 2008, 2012; Johnson et al., 2007; Romer, McConnell, & Jones, 2002a), however shorter interventions are sufficient to increase $P_{Imax,}$ P_{oes} and P_{di} following four weeks IMT (Brown et al., 2014). Durations of four weeks are sufficient to elicit positive adaptations in inspiratory muscle function; Romer and McConnell (2003) identified that 6 weeks of training maximises the opportunity for adaptation as almost all of the pressure-flow adaptations are expressed by this point.

Additional improvements in the pressure-flow characteristics of the inspiratory muscles have been observed to coincide with increased optimal inspiratory pressure (25%), optimal inspiratory flow rate (17%) and the maximum rate of pressure development (18%, Romer et al., 2002). Also as discussed in greater detail in Section 1.3.2.3, PTL IMT has also observed increased diaphragm thickness (Downey et al., 2007) and changes in the proportion and size of type I and type II muscle fibres (Ramirez-Sarmiento et al., 2002). PTL IMT however has weak external validity as it bears little relation to dynamic inspiratory muscle function that is observed during whole-body exercise tasks, but it is cost effective, easy to use, and is less time consuming compared with FRL and VIH methods of RMT with each session usually lasting ~5 min. Moreover it has been used extensively within the literature and shown to provide an ergogenic effect to whole body performance in a multitude of testing modes and exercise modalities.

1.3.2.5 FUNCTIONAL INSPIRATORY MUSCLE TRAINING

A novel training technique was proposed by (Tong, McConnell, et al., 2014) who investigated the effects of functional inspiratory muscle training (IMT_F) and core muscle training on running performance which was assessed using a 60 min treadmill run. The first 30 min had fixed intensity at 65% $\dot{V}O_2$ peak and the second 30 min was free running where

the participants attempted to cover the greatest distance. Following a 4 week phase of foundation IMT which all participants conducted to control for the established ergogenic effects of IMT and to also prepare a strong foundation within the inspiratory musculature during subsequent IMT_F sessions (Tong, McConnell, et al., 2014). Participants were then randomly assigned into two groups and completed a further 6 weeks of specific interval training and either IMT maintenance or IMT_F, which consisted of four inspiratory loaded core exercises, performed immediately after each interval session. Following 4 weeks IMT Tong, McConnell, et al (2014) observed a 21% increase in P_{Imax} (P<0.05) which was further increased (7%), albeit non-significant (P>0.05, d= 0.40). There were no changes during the IMT maintenance phase. It is suggested here that IMT_F may supersede the ergogenic effect of traditional IMT methods as the body positions adopted during the training activities are designed to mimic those of exercise and provide a training stimulus to this muscle group. This form of training will therefore target the coordination of breathing and stabilisation of the trunk, which utilise similar muscles (Boussana et al., 2003; Tong & Fu, 2006) and establishes an important relationship during whole body exercise. It is attractive to suggest that this extension of traditional PTL IMT methods may provide further and more specific benefits to performance but this has yet to be understood in its entirety and warrants further investigation.

1.3.3 Effects of RMT on exercise performance

The use of RMT has been shown to provide an ergogenic effect to whole body performance within a host of population's including those with; reoccurring lower back pain, wheelchair users, respiratory health conditions and the elderly (Gosselink, Kovacs, Ketelaer, Carton, & Decramer, 2000; Janssens et al., 2015; Ramirez-Sarmiento et al., 2002). Specific studies that have looked to improve exercise performance in healthy individuals will be

reviewed here due to the lack of studies that have assessed the effectiveness of RMT programmes within an occupational context. It is well documented that adopting any of the aforementioned techniques that RMT does not increase $\dot{V}O_2$ peak as RMT sessions do not pose a sufficient stimulus to any component that determines peak O_2 uptake, where: $\dot{V}O_2$ peak = $\dot{Q}$ max × a-vO_2diff (HajGhanbari et al., 2013; Romer et al., 2002a). To date there is only one study that has investigated the use of RMT techniques to improve performance on occupational tasks (outlined later within this Chapter, Section 1.4.4.3). It was demonstrated here that FRL IMT had no effect upon unloaded incremental exercise performance in members of the German Special Forces (Sperlich, Fricke, De Marées, Linville, & Mester, 2009). It is imperative to note here, that this study failed to observe an increase in P_{Imax} post-RMT; no observed training effect was noted which is most likely because breathing pattern was not controlled during the training and therefore nor was training intensity. To date there are no further studies to use military personal or to assess the effectiveness of RMT interventions on exercise performance therefore, literature from healthy individuals and exercise trials must be reviewed.

Leddy et al., (2007) assessed the effectiveness of a 3 month VIH intervention on running performance in endurance runners and using a 4 km time-trial exercise task. This is a logical comparison to make due to the frequency of running tasks that occur in occupational roles (Knapik, Harman, Steelman, & Graham, 2012). Leddy et al., (2007) observed no change in P_{Imax} or P_{Emax} post intervention but did observe a 4% (P<0.05) reduction in the time to complete a 4 mile time-trial run and sustained respiratory endurance. There are no other studies to investigate running performance using a time-trial performance measure but several studies have investigated the effects of RMT on intermittent running performance. Tong et al., (2008) was the first of these and separated two repeat Yo-yo intermittent recovery shuttle tests with a 6 week period of RMT consisting of, 6 sessions of PTL training over a 6 week period

with the device set at 50% P_{Imax}, completing 30 inspiratory efforts from residual volume twice daily. P_{Imax} was significantly increased (P<0.05) in the training group only and a group mean change of 17% (P<0.05) was observed in favour of RMT following the intervention to coincide with reduced perceptions of effort (Tong et al., 2008). The findings of this study have since obtained support from other groups that have shown increased inspiratory muscle strength, attenuated respiratory muscle fatigue, reduced perceived exertion, reduced metabolic stress and improved performance on the Yo-Yo intermittent recovery shuttle run test (Lomax et al., 2011; Nicks, Morgan, Fuller, & Caputo, 2009). Reduced recovery time between repeated exercises has also been reported by Romer, McConnell, & Jones., (2002b) who instructed participants to self-select the recovery time, following 6 weeks RMT with 30 breaths twice daily at 50% P_{Imax} recovery time was reduced by 6 ± 1% in RMT only (P<0.05).

Cycling and rowing have also received considerable attention from RMT research; these sports pose an additional task to ventilation which may be more relevant to load carriage tasks. These activities pose restrictions to normal breathing mechanics whilst completing the sport specific actions (Boussana et al., 2003), and require increased recruitment of both obligatory and accessory muscles of inspiration due to the dual role the respiratory musculature in providing ventilation and stabilisation of the trunk (HajGhanbari et al., 2013). This combined with increased lumbar and thoracic flexion which reduces EELV and increases expiratory resistance and abdominal pressure, diaphragmatic work for given ventilatory rates and also the WOB (Boussana et al., 2003) which shifts inspiration to higher lung volumes (Tomczak, Guenette, Reid, McKenzie, & Sheel, 2011). Volianitis et al., (2001) first examined the effects of 6 weeks RMT upon 6 min all out rowing performance and in a separate 5 km rowing time trial performance. Participants completed 11 weeks of PTL IMT (30 breathes twice daily at 50% P_{Imax}) or PLA RMT (60 breathes once daily at 15% P_{Imax}). Post intervention, the time to complete 5 km time trial was reduced by 3.1% (36 s; P<0.05) and the

distance covered in 6 min was improved 3.5% (52 m; $P<0.05$) following RMT, there was no change following sham training. The findings here were not supported by a later study by Riganas et al., (2008), who prescribed the use of the PTL device for 0.5 hours, 5 times a week for 6 weeks at an initial intensity of 30% P_{Imax} and increasing by 5% following each session until 80% P_{Imax} was achieved. Despite a 28% increase in P_{Imax} there were no changes in 2000 m rowing performance, nor changes in $\dot{V}O_2$ peak, perceived exertion or blood lactate responses (Riganas et al., 2008). The use of VIH was adopted in this study which, as discussed previously in Section 1.3.2.2 targets increased respiratory muscle endurance however the data here demonstrates despite an increase in P_{Imax} similar respiratory muscle fatigue post intervention, potentially inhibiting improved performance. Improvements have also been reported in 20 (3.8%, $P<0.05$) and 40 km (4.6%, $P<0.05$) simulated cycling time-trial performance on a cycle ergometer (Romer et al., 2002b). Improvements here also included increases in P_{Imax} (28 ± 7%), relative reductions in respiratory muscle strength were attenuated post time-trial when compared with pre RMT values; there were no change in any values in PLA. This was furthered by Johnson et al. (2007) where participants' using their own bicycle mounted on an air-braked ergometer system completed a 25 km cycling time-trial either side of a 6 week IMT training intervention. Results here observed a 2.7% increase in time-trial performance to coincide with a 17.1% increase in P_{Imax}, with no change in a placebo group.

The use of expiratory muscle training (EMT) techniques have observed increased expiratory muscle strength, which may be of benefit to whole body performance. Derchak et al., (2002) observed increased MSNA with heightened expiratory muscle work that contributed to the onset of the respiratory muscle metaboreflex (Section 1.2.7). Therefore increasing the relative strength of this group, as detailed extensively for the inspiratory muscles, may also contribute to increased performance and reduced perceptual responses

during exercise. As stated previously, Griffiths and McConnell (2007) observed a significant 2.7% improvement in a 6 min all out rowing ergometer test following 4 wk IMT but observed no change in performance following 4 wk of EMT. To further this, a subsequent 6 wk period of combined IMT and EMT failed to further change any performance measures. Increasing the pressure generating capacity of the expiratory muscles has also proved problematic as increased gastric pressure increases the likelihood of expiratory flow limitation which promotes hyperinflation and markedly increases the work of breathing (Aliverti, 2008).

To summarise research, that has investigated the effects of IMT on whole-body exercise performance produce difficult interpretation of results, as they are confounded by the use of many different performance tests, differing modes of training, inappropriate sample sizes and a lack of appropriate controls. The use of well controlled placebo-controlled methodologies have demonstrated significant improvements in both cycling, rowing and running time-trial exercise performance as well as intermittent exercise performance. Collectively the findings here illustrate an ergogenic effect of IMT on whole body performance but question the use of EMT to further improve performance. RMT appears to provide an improvement in time-trial type exercise from 2 to 6 % (for a full review see HajGhanbari et al., 2013; Illi et al., 2012). Romer et al., (2002b) suggest that as the improvements in performance observed within literature exceed about half of the natural variance in human performance, the IMT-mediated improvements in exercise performance present a meaningful ergogenic effect which most importantly improves athletic performance. To date the use of a well-controlled study design has not been used to assess the effectiveness of an IMT when assessing performance on occupationally relevant tasks.

1.3.4 MECHANISMS FOR IMPROVED PERFORMANCE WITH RMT

The mechanism by which RMT improves physical performance remains largely unclear. It is understood however that the adaptations are not localised to the cardiovascular system with no change in arterial blood gases; for example RMT sessions pose only a moderate cardiovascular demand (Romer et al., 2002c). There is evidence to suggest the structural adaptations that occur specifically within the muscles and the neural interactions between the central and peripheral nervous systems are also altered. The key adaptations that have been observed within literature to date suggest that RMT seeks to increase respiratory muscle strength and subsequently attenuate the onset of respiratory muscle fatigue, sustain locomotor muscle perfusion through blunted sensory motor input, attenuate feelings of exertion and discomfort, promote a change in breathing mechanics and favourable reductions in perceived exertion.

1.3.4.1 ATTENUATION OF RESPIRATORY MUSCLE FATIGUE

Respiratory muscle fatigue is often measured by pre-post exercise reductions in P_{Imax} as described previously in Section 1.2.1, and these reductions have been attenuated following a period of RMT which has been demonstrated during intense rowing exercise (Griffiths & McConnell, 2007; Volianitis, McConnell, Koutedakis, McNaughton, et al., 2001), cycling time trials (Johnson et al., 2007; Romer et al., 2002b), incremental cycling performance (Bailey et al., 2010), intermittent running performance (Nicks et al., 2009) and intense constant power exercise in normoxia and hypoxia (Downey et al., 2007). Initial observations however of Verges et al (2006) demonstrate no reduction in respiratory muscle fatigue after constant-load high intensity endurance exercise at 85% peak power output. Despite an initial 272% improvement in respiratory muscle endurance, the findings here show that the pre and post intervention rates of fatigue were similar following endurance-focused respiratory

muscle training (VIH). Following sub-group analysis of the sample, reduced rates of fatigue were observed prompting the authors to suggest that the intervention was potentially ineffective in some participants, a notion that is discussed further in Section 1.3.5.

The resulting reduction in fatigue may be attributable to the increase in respiratory muscle strength which is prompted via structural adaptations within the respiratory muscles and are observed following IMT. To coincide with this, Enright et al., (2006) observed a ~10% increase in diaphragm thickness following 8 weeks of incremental respiratory endurance IMT consisting of 6 sets of 6 inspiratory efforts at 80% P_{Imax} with progressively reduced rest from 60 – 5 seconds on 3 non-consecutive sessions per week. This was supported by Downey et al., (2007) and more recently by Mills et al., (2015) who observed increased diaphragm thickness at end-inspiration (8.3% and, during a P_{Imax} manoeuvre 11.5%) and residual volume (38%) respectively. This coincides with increased P_{Imax} following a 4 (25%) and 8 week (34%) intervention. A strong relationship exists between the cross sectional area of a muscle and diaphragm hypertrophy is representative of a change in muscle fibre composition (Downey et al., 2007). Muscle biopsies taken from the human external intercostals post 5 weeks PTL IMT highlight increased proportion (38%) and size (21%) of type I and type II muscle fibres (Ramirez-Sarmiento et al., 2002). Studies within animals show an increase in diaphragm mitochondrial cytochrome-c oxidase activity observed following 3 weeks chronic FRL (Akiyama, Garcia, & Bazzy, 1996; Akiyama, Garcia, Prochaska, & Bazzy, 1994). Increased respiratory muscle oxidative capacity may reduce the reliance on/delay the recruitment of type II muscle fibres which are more susceptible to fatigue than their type I counterparts. It seems plausible that an IMT-mediated increase in the maximal force generating capacity of the respiratory musculature may reduce the absolute force generated for a given ventilation (Kellerman, Martin, & Davenport, 2000). This may

reduce afferent discharge to the sensory-motor cortex, thus attenuating a sympathetic-mediated efferent response (Witt, Guenette, Rupert, McKenzie, & Sheel, 2007).

1.3.4.2 ATTENUATION OF RESPIRATORY MUSCLE METABOREFLEX

As outlined in Section 1.2.7 fatiguing contractions of the respiratory musculature are associated with significant neural and cardiovascular consequences through a reflex mechanism known as the respiratory muscle metaboreflex and reduces arterial blood flow to the resting limb (Sheel et al., 2001; Witt et al., 2007). To date only two studies exist that have specifically addressed the effect of IMT on this reflex mechanism and illustrated an attenuation of the vasomotor consequences observed in the presence of respiratory muscle fatigue (McConnell & Lomax, 2006; Witt et al., 2007).

McConnell and Lomax (2006) demonstrated for the first time, and without any imposed limitation to $\dot{Q}$ due to the absence of dynamic exercise that inspiratory muscle fatigue induced via acute inspiratory loading at 60% P_{Imax} and a f_B of 15 breaths·min^{-1} prompted the onset of the metaboreflex and accelerated plantar flexor fatigue, which was observed via a 32% reduction in limb vascular conductance and is a finding that is in line with previous work (Sheel et al., 2001). Post 4 week IMT using a PTL device P_{Imax} was increased by 20.7% (P<0.05) and at the same relative intensity prior to IMT both inspiratory muscle fatigue was reduced by ~10% and the time to the limit of tolerance during plantar flexion exercise was significantly improved (McConnell & Lomax, 2006). The improvement in plantar flexion exercise tolerance observed here was attributed to an IMT-mediated improvement in limb vascular conductance following fatiguing inspiratory muscle work. This was furthered by Witt et al., (2007) who used 5 weeks PTL training at 50% baseline P_{Imax} and significantly increased baseline levels of respiratory muscle strength. The authors observed a reduction in a time dependant increase in HR and MAP and a reduction in afferent discharge

of chemically sensitive type IV muscle afferents when completing an identical resistive breathing task at 60% P_{Imax} pre and post IMT. The authors report a 17% increase in P_{Imax} post IMT which they suggest allows the respiratory muscles to work at a lower relative intensity (Turner et al., 2012) during the resistive breathing task. This therefore increases the threshold for respiratory muscle fatigue and reduces afferent discharge into the sensorimotor cortex (Sinoway et al., 1993) blunting the sympathetically mediated efferent response and ultimately sustaining limb vascular perfusion (Dempsey et al., 2006, 2002) and improving O_2 delivery to the locomotor muscles (Witt et al., 2007). The authors postulate that this is aided by structural adaptations outlined in the previous section that occur within the diaphragm and accessory muscle of inspiration, and increase the aerobic capacity of the respiratory musculature (Enright et al., 2006).

The use of PAV's to unload the respiratory musculature has been described in Section 1.2.7. Unloading the respiratory muscles during maximal exercise increased exercise performance by 14.4% on time to the limit of volitional tolerance (T_{LIM}) exercise tests, compared with subsequent loading trials that reduced exercise performance by 15.1% (Harms et al., 2000). In this study the WOB was unloaded by 37-45% during a cycling constant load exercise test at 90% $\dot{V}O_2$ peak and consequently reduced the $\dot{V}O_2$, hyperventilation response and perceptual responses, attributed to reduced respiratory muscle work and the effects on limb vascular resistance, suggesting the metaboreflex was attenuated with respiratory muscle unloading. The findings here were furthered by Romer et al., (2006) who also unloaded the WOB by 56% and saw a 33% attenuation of quadriceps muscle (m.quadricep) fatigue compared with a control trial. Similar reductions in $\dot{V}O_2$, limb discomfort and dyspnoea to that of Harms et al., (2000) were also observed during the unloaded trial and suggest that unloading the respiratory muscles during exercise improves exercise performance and may also attenuates the onset of the respiratory metaboreflex. This is of particular relevance as the

mechanisms here will be similar to that provided via IMT which may provide an unloading stimulus due to the increased absolute strength that occurs. This combined with increased absolute strength and structural adaptations; may reduce the relative WOB for given ventilatory rates prompting increases in the provision of energy through aerobic pathways. This results in a reduction of accumulating and fatiguing metabolites derived through anaerobic pathways. This in turn reduces discharge frequency of the chemically sensitive group IV muscle afferents (Dempsey et al., 2006; Witt et al., 2007) and reduces sympathetic nerve activity that projects to the sensorimotor cortex and attenuating changes to motor outflow and sustaining limb vascular conductance (Grippo et al., 2010; Witt et al., 2007). The attenuation of the metaboreflex following IMT has yet to be investigated under the conditions of dynamic exercise and therefore warrants further investigation. To further this, typically the onset of RMF is seen at constant load exercise at intensities greater than that of 85% $\dot{V}O_2$ peak. It is therefore likely that improvement in performance at sub-maximal exercise intensities is a result of other mechanisms which may include RMT-mediated changes in breathing mechanics and altered sensations of perceived exertion.

1.3.4.3 PERCEPTUAL RESPONSES

An increase in respiratory muscle strength reduces the relative work done by the respiratory musculature during exercise (McConnell, 2009) and as a consequence, whole body performance is increased through reductions in perceptual strain during exercise tasks (HajGhanbari et al., 2013). As described in Section 1.2.6 and in a recent systematic review by HajGhanbari et al., (2013), excessive perceptual strain linked to both dyspnoea and also whole body discomfort experienced during exercise tasks is a common cause for the termination of exercise but RMT has consistently demonstrated an attenuation of perceptual responses (Bailey et al., 2010; Griffiths & McConnell, 2007; Kilding, Brown, & McConnell,

2010; Romer et al., 2002b; Tong et al., 2008). This reduction in perceived effort can be explained in principle by an alteration in the neurophysiological mechanisms that seek to modulate perceptual responses (McConnell, 2009). Sensory areas of the brain compare the neural drive sent to working muscles against returning afferent information relating to generated tension during contraction, formulating a perception of effort (Amann, 2012; Cafarelli, 1981). This is applicable to all skeletal muscles and in the presence of respiratory muscle fatigue and during fatiguing contractions the neural feedback to the sensorimotor cortex is elevated through a time dependent increase in MSNA via increased group III and IV discharge, thus prompting an increase in perceived effort (Amann, 2012; Romer & Polkey, 2008). Increasing the strength of the respiratory muscles via RMT reduces the neural drive required to generate a given force as the level of force requires a lower proportion of its maximum capacity (McConnell, 2009), ultimately reducing MSNA and attenuating increasing perceptual responses (Sinoway et al., 1993). There is a multitude of research to show that RMT reduces the perceptions of effort experienced during endurance and high intensity exercise (Bailey et al., 2010, 2010; Romer et al., 2002b; Tong et al., 2008; Volianitis, McConnell, Koutedakis, McNaughton, et al., 2001).

It is important to note here that the perceptual responses are not localised to the respiratory musculature prompting changes in dyspnoea, recent evidence suggests that limb discomfort induced by fatiguing knee extensor exercise heightens the perception of breathing discomfort with no change in pulmonary ventilation (Grippo et al., 2010; Sharma et al., 2015). The afferent nerve endings found within the diaphragm and the respiratory musculature are also present within locomotor muscles and respond identically to fatigue and accumulating metabolites through increased projection of afferent discharge via the dorsal horn to the sensorimotor cortex (Dempsey et al., 2014). This increases both limb and breathing discomfort (Grippo et al., 2010; Sharma et al., 2015) and increases conscious

sensations of discomfort which serve as a limiting factor for whole body exercise performance (Amann, 2012; Harms et al., 2000; Romer & Polkey, 2008). Therefore, improved locomotor muscle function following RMT due to improved limb blood flow through an attenuation of the metaboreflex would also seek to improve performance.

1.3.4.4 REDUCED ACCUMULATION OF BLOOD LACTATE

Increased exercise performance may derive from a reduction in circulating metabolites that may attenuate the discharge of chemo-sensitive afferent fibres (Type IV) located within the diaphragm and is a known trigger of the respiratory muscle metaboreflex (Rodman, Henderson, Smith, & Dempsey, 2003). Rodman et al., (2003) observed increased arterial blood pressure and reduced $\dot{Q}$ in the hind legs of dogs by 21% and 6% respectively with the use of lactate infusions into the phrenic or iliac arteries of awake dogs; these were attenuated by an adrenergic receptor blockade at rest. The authors conclude here that accumulation of circulating metabolites (lactate and hydrogen ions) constitutes the onset of the respiratory muscle metaboreflex contributing to increased vascular resistance within the exercising limb (Rodman et al., 2003).

Reductions in blood lactate ($[Lac^-]_B$) after RMT is a common observation and have been reported at rest during volitional hyperpnoea following RMT (Brown, Sharpe, et al., 2008; Spengler, Roos, Laube, & Boutellier, 1999), during cycling exercise at maximal lactate steady-state during both with and without superimposed VIH (Brown et al., 2012; McConnell & Sharpe, 2005), 6 min maximal rowing (Griffiths & McConnell, 2007; Volianitis, McConnell, Koutedakis, McNaughton, et al., 2001), during repeated sprint exercise (Romer et al., 2002b; Tong et al., 2008) and constant power exercise until the limit of tolerance (Bailey et al., 2010; Boutellier & Piwko, 1992; Leddy et al., 2007). Reduced $[Lac^-]_B$ may occur following RMT as the musculature increase their lactate uptake and metabolism or decrease

efflux, or possibly a combination of both (Brown et al., 2012; Spengler et al., 1999). To further this, Romer et al., (2002b) observed a significant correlation between the change in systemic $[lac^-]_B$ and recovery time taken between repeated sprints as well as the reduction in perceptual responses.

During sub-maximal exercise the adaptations in type I muscle fibre composition and oxidative processors that occurs as a result of RMT that are described in Section 1.3.6.1, could result in prolonged utilisation of aerobic pathways during exercise thus reducing the concentration of circulating metabolites (Sinoway et al., 1993). During maximal and fatiguing exercise a chronic adaptation to respiratory muscle training may see the sensory afferents desensitised to the accumulation of fatiguing metabolites (Dempsey & Smith, 2014; Witt et al., 2007). Repeated exposures to high metabolite concentrations that occurs within RMT sessions would blunt the afferent-mediated efferent response to a given change in metabolite concentration during subsequent exercise (Sinoway et al., 1993), resulting in reduced perception of effort and sustained central motor drive and limb vascular conductance.

1.3.5 CONSIDERATIONS FOR RMT STUDY DESIGNS

The findings of RMT techniques on performance have elicited inconsistent improvements on athletic performance. A review by HajGhanbari et al., (2013) attributed such inconsistencies amongst research to a three key factors; small sample sizes, individual responses and the methods of RMT used within a study design. The use of small sample sizes within studies may inhibit the detection of small/moderate improvements on performance tasks. Sample size calculations derived from this review suggest that to detect moderate and large effects, each group within a randomised control trial would require 64 and 26 participants respectively to achieve the statistical significance with a priori alpha of 0.05. Future studies however should not base sample size calculations upon the suggestions of a

review of numerous randomised control trials but should each conduct a reliability analysis of their protocol to understand the random and systematic error of performance measure and using these to determine the appropriate sample sizes to minimise these. To further this, and in acknowledgement of the strengths of some research designs, those that return significant and interaction effects against a placebo control with matched groups permits the statistical analysis to account for potential error and variation (Hopkins, 2000).

One such consideration here maybe the lack of observed significance as a result of individual differences, as achieving statistical significance is reliant on the presence of a substantial benefit for all participants within the group (HajGhanbari et al., 2013). The presence of non-responders or those that respond less favourably to methods of RMT within a sub-group of participants may seek to lessen the observed effect and reduce observations of increased performance. This is true of Verges et al., (2007) who employed VIH to improve respiratory muscle endurance in a constant load cycling test at 85% max peak power output (PPO). Initial results observed no change in respiratory muscle endurance but further analysis of a sub-group of participants who had a >10% reduction in trans-diaphragmatic pressure revealed an attenuation of exercise induced reductions in trans-diaphragmatic pressure post VIH and not post CON (Verges, Lenherr, et al., 2007). Controlling for responders and non-responders within participant samples is difficult and appropriate sub-group analysis can be used where necessary to highlight the extent of changes, but careful selection of RMT methods and regimes that have shown to broadly elicit positive adaptions may account for within sample differences and allow greater observation of differences within performance (HajGhanbari et al., 2013). Matching participant groups for physical fitness and similar levels of respiratory muscle strength may also prove advantageous, as those of lower physical fitness appear to benefit more from a period of IMT (Illi et al., 2012). Brown et al., (2014) however observed individual and within group differences in both respiratory and ventilatory

parameters despite groups being matched for age, height, weight (and other characteristics that affect may ventilation). This consideration however may be negated as changes in respiratory muscle strength are not correlated with changes in performance on exercise cycling tasks (Johnson et al., 2007).

There is also a need to select the most appropriate method of RMT for interventions within a study design. To further this and as outlined in Section 1.3.2, traditional techniques that seek to target the force-velocity relationship and knowledge of the ventilatory demands imposed during performance measures should be considered when selecting the appropriate RMT method. HajGhanbari et al., (2013) and Illi et al., (2012) state that the method of RMT should due to the training specificity principles replicating the demands experienced during exercise in order to elicit the most positive adaptations. An important second training principle is that RMT training programmes contain an overload stimulus to promote adaptation and within training there should be progression as to sustain the overload stimulus and permit further progression (HajGhanbari et al., 2013; McConnell, 2009). Other factors associated with study design highlight the lack of investigator blinding and concealed allocation within previous research that has used randomised control study designs. The inclusion of a sham-training group was present in almost half of the RMT studies used in a review by HajGhanbari et al., (2013) and the use of such groups to account for a possible placebo affect should be integrated into RMT studies to act as a marker for the effectiveness of an intervention (HajGhanbari et al., 2013; Illi et al., 2012). Of the studies that employed a sham-placebo, single blinding was common to protect the participants from bias within the study. Investigator blinding is rare and guidance form Schulz, Altman, Moher, & others, (2010) suggest placebo designs should be a key consideration in RMT studies that adopt a randomised control trial design to prevent bias on behalf of the investigator.

1.4 LOAD CARRIAGE

Load carriage is defined as "locomotion whilst transporting an external mass supported on the upper torso by shoulder straps and/or hip belts" (Knapik, Harman, & Reynolds, 1996a, p. 208) and has relevance to individuals that are employed in physically demanding occupations such as the military and those that enjoy recreational tasks such as hiking (Birrell & Haslam, 2010). Prior to the 18th century, military personnel rarely carried loads in excess of 15kg due to the use of auxiliary transport (horses, carts, assistants etc.) however towards the turn of the 19th century, structural changes saw soldiers carrying their own equipment (Knapik & Reynolds, 2010). Since then, technological advances in both weaponry and bodily protection have been essential in aiding soldier safety and firepower leading to a dramatic increase in the weight of the loads being carried (Knapik et al., 1996). The typical weight that is carried by soldiers often exceeds 30-40% of body mass, but has been reported to extend as far as 70% (Grenier et al., 2012) representing extreme loads. In recent conflicts, soldiers on deployment in Afghanistan and Iraq wore loads that amassed up to 75kg (Knapik et al., 2012). There are various methods posed within research when carrying loads, however it often takes the form of a backpack which is especially common in instances where the use of vehicles is restricted due to the demands of the terrain or passive noise might jeopardise specific tasks (Knapik et al., 2012).

1.4.1 METHODS OF LOAD CARRIAGE

The need to research methods of load carriage was stimulated due to an increase in the number of musculoskeletal joint injuries and the performance limiting characteristics of load carriage that have been observed in research using load carriage systems (Holt, Wagenaar, Kubo, LaFiandra, & Obusek, 2005). As stated in the previous section, military loads are commonly worn in the form of a backpack (Knapik & Reynolds, 2012) and are conveniently

organised to satisfy operational demands such as the need to quickly access battle equipment (munitions, weapons, and electronics that are typically located on the front of the trunk) and benefit from a large protection against enemies and environments (helmet, joint protections, and heavy boots typically located in distal regions of the body). These loads provide supplies and supplementary equipment that are typically located in an additional backpack that, represents a distinct and complex load distribution compared to backpack carriage only (Grenier et al., 2012).

Research in Nepalese women identified that the most economic method of load carriage occurs when the load is positioned above the head and directly above the centre of mass. This causes little biomechanical and physiological adjustments to accommodate the load (Knapik et al., 2012). This method lacks practicality and has reduced functional capacity during recreational and occupational tasks for the military where loads are carried upon the thorax using a backpack (Figure 1.18, Knapik & Reynolds, 2012). The bearing of external loads in a backpack is of equal convenience and efficiency which serves as rationale for its widespread use within occupational and recreational groups as detailed previously.

CONTENT REMOVED FOR COPYRIGHT REASONS

See the following reference for full image:

Knapik, J., & Reynolds, K. (2010). Load Carriage in Military Operations: A review of historical, physiological, biomechanical and medical aspects (p. 78). Borden Institute.

Figure 1.18 A representation of a soldier wearing a full complement of military combat clothing (A) and a schematic of load carriage and the fixings of a backpack to the body (B), Picture adapted from Knapik & Reynolds, 2010).

As the use of backpacks is common place in these groups, the focus of research has since changed to allow greater understanding of how the placement of the load within a load carriage system affects physiological and biomechanical systems, which is an important factor in reducing energy cost (Abe, Muraki, & Yasukouchi, 2008b; Knapik & Reynolds, 2012) and increasing stability (Simpson, Munro, & Steele, 2011). It is well conceived that placing the load high in the pack, close to the packs frame and the centre of mass of the body is the most efficient place to reduce physiological and biomechanical stress upon the body (Knapik, Reynolds, & Harman, 2004; Legg & Mahanty, 1985) as demonstrated in Figure 1.19.

CONTENT REMOVED FOR COPYRIGHT REASONS

See the following reference for full image:

Knapik, J., & Reynolds, K. (2012). Load carriage in military operations: a review of historical, physiological, biomechanical, and medical aspects. Military Quantitative Physiology: Problems and Concepts in Military Operational Medicine, 303.

Figure 1.19 Shows the placement of a load within a backpack close to the frame of the pack providing optimal energy efficiency during loaded exercise (Knapik & Reynolds, 2012).

1.4.2 PHYSIOLOGICAL RESPONSES TO LOAD CARRIAGE

Research investigating the physiological responses to load carriage tasks has typically been conducted with exercise durations less than 15 min. Again this is not a true reflection of

exercise duration in recreational and occupational settings which can range from 30 min to several hours (Brown & McConnell, 2012a; Grenier et al., 2012) and causes additional and important physiological responses in comparison to shorter durations. This includes $\dot{V}O_2$ and heart rate during exercise that increase proportionally with load mass (Blacker, Fallowfield, Bilzon, & Willems, 2009), however, this information is not reflective of occupational demands as exercise duration is typically <30 min. For the purpose of this thesis, the duration of prolonged exercise is defined as >30 min and up to durations of 240 min. Literature has observed increased heart rate during bouts of 145 min of load carriage with 49.4 kg at 50.1% $\dot{V}O_2$ peak which continues to increase throughout the duration of exercise which is indicative of cardiovascular drift (Patton, Kaszuba, Mello, & Reynolds, 1991). Epstein et al., (1988) showed that when a 40 kg load was carried in a backpack at 4.5 km·h^{-1} on a +5% gradient for 120 min $\dot{V}O_2$ increased between 20 and 120 minutes from 52.1 ± 0.6 to 56.2 ± 0.6% $\dot{V}O_2$ peak respectively, although these observations were not apparent when carrying a 25 kg load under the same conditions. The observations here suggest that $\dot{V}O_2$ drift occurred when the work rate was increased above 50% $\dot{V}O_2$ peak. These findings have since been confirmed by Blacker et al., (2009) where participants familiar with load carriage activities through recreational and occupational tasks completed 120 min of treadmill walking at 6.5 km·h^{-1} with a 25 kg load and compared to an identical trial with no load. This comparison showed that $\dot{V}O_2$ was 41 ± 17% (23.0 ± 2.7 vs. 16.4 ± 0.7 ml·kg^{-1}·min^{-1}, P<0.01) greater after 5 min and continued to increase to 50 ± 19% $\dot{V}O_{2max}$ at minute 120 (26.9 ± 3.3 vs. 17.9 ± 0.5 ml·kg^{-1}·min^{-1}, P<0.01, Figure 1.20). HR during the load carriage trial was 25 ± 7 % higher at minute 5 compared with the unloaded trial (116 ± 13 vs. 93 ± 8 beats·min^{-1}, P<0.01) and 43 ± 16 % higher at minute 120 (141 ± 23 vs. 99 ± 12 beats·min^{-1}, P<0.01). There was also a greater increase in HR over the 120 min during the loaded trial (116 ± 13 to 141 ± 23 beats·min^{-1}, P<0.05) compared to the unloaded trial (96 ± 10 to 99 ± 12 beats·min^{-1}, P<0.05). The loaded

trial also elicited higher lactate and glucose concentrations, increased perceptual responses and stride frequency and reduced stride length when compared with the unloaded trial (P<0.05).

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See the following reference for full image:

Blacker, S. D., Fallowfield, J. L., Bilzon, J. L., & Willems, M. E. (2009). Physiological responses to load carriage during level and downhill treadmill walking. Medicina Sportiva, 13(2), 116–124.

Figure 1.20 $\dot{V}O_2$ from baseline during 120 min of treadmill walking at 6.5 km·h^{-1} when walking with no load (squares) and with a 25 kg backpack (triangles), (edited from Blacker et al., 2009).

The findings here support the notion of a cardiovascular and $\dot{V}O_2$ drift when carrying no load which is not surprising considering the cardio-respiratory interactions, as outlined in Section 1.27. The increase in $\dot{V}O_2$ is explained by increased muscle fibre recruitment which subsequently increases the demand for O_2 therefore causing $\dot{V}O_2$ to rise. The observed $\dot{V}O_2$ drift during loaded walking may be caused by the prolonged exercise where muscle fibres become fatigued and/or damaged, reducing the force they are able to produce (Millet & Lepers, 2004). To accommodate for this, additional motor units are subsequently recruited to maintain movement on the treadmill at the required speed and to support the load. This additional recruitment will increase O_2 demand thus increasing $\dot{V}O_2$ and prompting $\dot{V}O_2$ drift

(Blacker et al., 2009). The extent to which changes in physiological markers are affected by load carriage has also highlighted an increase in energy expenditure and a ~20% decrease in physical capabilities (Knapik & Reynolds, 2012).

1.4.2.1 ENERGETICS OF LOAD CARRIAGE

The energetics of load carriage has received considerable attention from research that has investigated energy expenditure when marching both with and without loads and identified an increase of ~10% (Knapik et al., 1996). Bastien et al., (2005) reported a gross increase in the cost of walking with a load at speeds of 1.1m·s^{-1} (3.96 km·h^{-1}) with intermediate (30% - 45% of body mass) and high-end load carriage (75% of body mass). The results here were consistent with existing non-military literature as they pinpointed the net increase of energy expenditure at 2.4 J·kg^{-1}·m for 30% and 45% of body mass and the gross cost of walking with a load of 75% body mass was 3.6 J·kg^{-1}·m. Grenier et al., (2012) reported findings that are in agreement with Bastien et al., (2005) as they investigated the metabolic cost of walking unloaded, with military battle equipment (mass= 22.4 kg ±1.1, 27.2% ±1.9 of mean body mass) and road marching equipment (mass = 37.9 kg ±1.4, 46.1% ±3.6 of mean body mass). Their findings suggested a significant increase of 43% in the metabolic cost of walking with increases in the load being carried. The increase in metabolic activity and energy expenditure during load carriage activities is proportional to the increase in $\dot{V}O_2$. Epstein, Rosenblum, Burstein, & Sawka., (1988) reported an initial increase in exercise intensity of 45.5 ± 0.6 % $\dot{V}O_2$ peak after walking for 20 min, which did not change over the 120 mins of exercise when carrying a 25 kg load in backpack when walking at 4.5 km·h^{-1} with +5% gradient. However carrying a 40 kg load for 20 min resulted in an initial intensity at of 52.1 ± 0.6 $\dot{V}O_2$ peak which subsequently increased to 56.2 ± 0.6 $\dot{V}O_2$ peak at

120 min. It was concluded here that exercise elicits a response >50% $\dot{V}O_2$ peak and causes a time dependent increase in $\dot{V}O_2$ peak that increases energy expenditure.

Autonomous changes in biomechanical and kinematic parameters have been observed where altered gait parameters and more specifically reduced stride length and increased stride frequency (Birrell & Haslam, 2010; Simpson et al., 2011) occur when exercising with thoracic loads. This coincides with increased flexion at the hip (Heller et al., 2009), which is necessary to compensate for increased ground reaction forces that are proportional to the mass of the load (Birrell, Hooper, & Haslam, 2007) and to protect the musculature of the thigh from excessive forces. This results in muscle damage and neuromuscular impairment (Attwells, Birrell, Hooper, & Mansfield, 2006) and attempts to protect from excessive metabolic and physiological changes in an attempt to sustain homeostasis. It is suggested here that the metabolic cost of walking whilst carrying a load does not increase with loads that are below 20% of the body mass of the person carrying the load (Abe, Muraki, & Yasukouchi, 2008a). This has been termed the *'free-ride* hypothesis*'* and is explained via the interaction between both the rotative torque and the excessive burden on the lower leg extremities caused by the presence of the load. Wearing the load within a load carriage system as detailed above is the most efficient of placements which reduces energy expenditure and subsequently increases rotative torque. This contributes to forward propulsion of the body by increasing anterior pelvic tilt pushing the body in a lateral direction (Abe et al., 2008a) as demonstrated in Figure 1.21.

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See the following reference for full image:

Abe, D., Yanagawa, K., & Niihata, S. (2004). Effects of load carriage, load position, and walking speed on energy cost of walking. Applied Ergonomics, 35(4), 329–335.

Figure 1.21 Diagram showing the interaction between the rotative torque operating around the body's centre of mass and the associated burden on the lower leg muscles during walking with load on the back. A= centre of mass, B= centre of mass of the load, AB = radius of rotation, C= rotation arc, D= associated burden on the legs, E= rotative torque functioning around the centre of body mass (Abe, Yanagawa, & Niihata, 2004).

The exercise intensity undertaken with thoracic loads has also attracted attention from research. Legg and MaHanty., (1985; 1992) observed no differences in metabolic cost when carrying loads in five differing methods close to the trunk when walking at 4.8 $km \cdot h^{-1}$ with a backpack weighing 26 kg. The authors concluded that any physiological differences were likely to occur when carrying loads in particular regions of the body and suggested further research was warranted to examine this idea. The findings here conflicted with Abe et al., (2008a) who observed a 'U' relationship between the speed and metabolic cost of walking (Figure 1.22) during walking with load on the upper back (in a backpack) compared with carrying a load on the lower back and a control measure with no load at differing walking speeds (30 - 130 $m \cdot min^{-1}$, 1.8 - 7.8 $km \cdot h^{-1}$). The authors concluded that the cost of walking was significantly reduced when carrying external loads on the upper back in comparison to the lower back (Figure 1.22) when walking at speeds of 60-80 $m \cdot min^{-1}$ (3.6 - 4.8 $km \cdot h^{-1}$).

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See the following reference for full image:

Abe, D., Muraki, S., & Yasukouchi, A. (2008a). Ergonomic effects of load carriage on energy cost of gradient walking. Applied Ergonomics, 39(2), 144–149.

Figure 1.22 Metabolic cost of walking in three conditions as reported by Abe et al., (2008a), U = load placed on the upper back, L = load placed on the lower back and C= Control condition with no load.

1.4.2.2 SKELETAL MUSCLE FATIGUE

As a result of prolonged engagement in load carriage activities in occupational settings that can total numerous hours, and with the imposed physiological demand, previous research had also focused on changes in force producing capacity of the skeletal muscles immediately after and in subsequent days of load carriage. Prior to the work of Blacker et al., (2009; 2010, 2013), methods used to assess decreases in neuromuscular function following load carriage were basic with little or no attention paid to the reliability and familiarisation of participants. More sophisticated methods have been used to investigate the severity and mechanisms involved in the changes in neuromuscular function following load carriage exercise (Blacker, Fallowfield, Bilzon, et al., 2013). Reductions in the force producing capability of a muscle has negative neuromuscular and metabolic consequences (Byrne, Twist, & Eston, 2004),

therefore, if neuromuscular function is compromised in the days following load carriage, an individual's physical performance is likely to be impaired. This has particular relevance in occupational settings as, following load carriage, participants are often required to undertake additional physically demanding and skilled tasks such as setting up and use of equipment and military specific tasks (Knapik et al., 2004) or additional bouts of load carriage (Blacker, Fallowfield, Bilzon, et al., 2013). During human locomotion and load carriage activities, the basic muscle function is the stretch shortening cycle, where the pre-activated muscle is first stretched (eccentric action) and then followed by the shortening (concentric) action. Neuromuscular impairment is greatest following eccentric contractions, the pattern of which has been suggested to be bimodal (Dousset et al., 2007). Typically there is an immediate reduction in the force production of a muscle, with a small recovery within one-two hours that is followed by a secondary reduction, where recovery can last for between four to eight days depending on the severity of the exercise bout (Dousset et al., 2007).

Blacker et al., (2013) investigated changes in neuromuscular function following 120 min of load carriage during level treadmill walking whilst also investigating the changes using voluntary and electrically stimulated contractions 0, 24, 48 and 72 h after load carriage. During an unloaded control trial, no changes in neuromuscular function were observed after walking for 120 minutes at 6.5 $km \cdot h^{-1}$ on a 0 % gradient. However, the addition of carrying a 25 kg backpack caused reductions in the levels of force produced by the knee extensors during both isokinetic and isometric contractions; findings were still apparent up to 72 h following the load carriage bouts. This is of particular importance considering the role of the respiratory muscle metaboreflex described in Section 1.2.7, where it is suggested that subsequent changes in sympathetic outflow and limb vascular resistance that occur in the presence of respiratory muscle fatigue reduce O_2 delivery to the locomotor muscles thus

prompting fatigue. This remains purely speculative though as exercise with thoracic load carriage and the effect this has on respiratory function is unknown and yet to be investigated.

1.4.3 LOAD CARRIAGE AND THE RESPIRATORY SYSTEM

A recent review by Brown and McConnell (2012) acknowledged that thoracic load carriage induces volume limitation upon the thorax which increases the elastic work of breathing, rendering the respiratory muscles vulnerable to fatigue, but little is known about the extent to which thoracic loads reduces both respiratory and exercise capacity. The consequences of load carriage and the effect this has upon respiratory function are unknown and the current understanding is derived from research that has outlined the effects of resting pulmonary function, where studies have used inelastic strapping and occupational equipment (e.g. self-contained breathing apparatus, SCBA).

1.4.3.1 RESTING RESPONSES TO PULMONARY FUNCTION

The effects of load carriage on a host of physiological variables have been investigated to understand the affect that load carriage has on the bodies' physiological responses. Dominelli, Sheel, & Foster., (2012) for example reported a significant change in a number of pulmonary measurements as a result on incremental loading with a backpack. They also reported the negative effect that this has on the function of the respiratory system as breathing mechanics become impaired and the negative effect this on resting pulmonary function. A 12% reduction in forced vital capacity (FVC) and forced expired volume in one second (FEV_1) when wearing a 25 kg backpack was observed during resting conditions. Reductions in vital capacity (VC) are not observed but, during periods of high intensity work, the ventilatory demand is increased 10-15 fold which has detrimental effects on the ventilatory reserve. During normal respiration, V_T increases by a decrease in end-expiratory lung volume (EELV), which serves to optimise diaphragm length (Aliverti, 2008). A further

consequence of loading the thorax with an external load is the restriction of the chest wall which further impedes operational lung volumes and hinders operating function of the respiratory system. This results in increased demand of the inspiratory muscles contributing to the premature development of respiratory muscle fatigue, caused by the presence of a backpack which imposes an inspiratory volume limitation of the thorax, thus impairing the function of the respiratory system during resting conditions (Brown and McConnell, 2012). This understanding has been furthered during dynamic exercise where the use of thoracic restriction and occupationally relevant equipment (breathing apparatus) has been used.

1.4.3.2 EXERCISE WHEN WEARING A THORACIC LOAD

Self-contained breathing apparatus (SCBA) systems are common place in occupational groups (i.e. firefighters) and are used to protect the respiratory system during occupational tasks as it reduces maximal ventilation. These systems, typically worn upon the thorax, which pose a limitation to ventilation and alter breathing mechanics which utilises a great proportion of EILV and increase the elastic WOB at rest and during sub-maximal exercise at 240 w (~59%); no difference was observed at exercise intensities <240 w. The exact mechanism is not known but it proposed that the presence of a positive pressure surrounds the face mask which adds a resistive load to breathing (Butcher et al., 2006). This increase in the WOB exacerbates the dyspnoea response to exercise with SCBA and limits exercise performance during short duration (~5 min) exercise tasks (Butcher et al., 2006; Eves, Jones, & Petersen, 2005). The use of a low density gas mixture that comprises 21% O_2 and 79% helium (Heliox), providing a passive benefit to performance by reducing air flow turbulence during high exercise intensities that have increasing flow rates. This partially unloads inspiratory work and reduces the level of observed RMF following submaximal

exercise with SCBA (Butcher, Jones, Mayne, Hartley, & Petersen, 2007a; Eves, Petersen, & Jones, 2003).

Chest wall restriction has also been used (using inelastic strapping) to assess the cardiorespiratory function at rest and during submaximal cycling at 25% and 45% PPO (Miller et al., 2002). At rest, TLC was decreased by 33%, VC was decreased by 38%, RV was also decreased by 23% and both peak expiratory and peak inspiratory flow rates were significantly reduced (P<0.05) during CWR conditions. CWR also resulted in reduced V_T and increased f_B to coincide with increased work done by the diaphragm, which saw the biggest rise in P_{ga}. This led to reductions in $\dot{Q}$ during exercise (~10-12%) a result of reduced SV (~16%) as HR was unchanged. The reductions in SV remain purely speculative but it is suggested that imposed reductions in $\dot{Q}$ is a result of increased intra-thoracic pressures swings and elevated P_{ga} caused by the restriction which reduces venous return and blood flow through the vena cava; thus reducing the pre-load and subsequently SV and $\dot{Q}$ (Miller et al., 2002). The use of inelastic strapping further demonstrated specific fatigue of the diaphragm in a study similar to that of Miller et al., (2002) which similarly used thoracic restriction during sub-maximal cycling exercise at 45% PPO (Tomczak et al., 2011). Inelastic straps were used to restrict FVC to 40% and diaphragm contractions were measured using cervical magnetic stimulation using both potentiated and non-potentiated twitches, for 10 and 30 min post exercise which was matched for duration. During exercise with inelastic strapping, the work of breathing, respiratory pressures, ventilatory parameters (Figure 1.23), and perceptions of respiratory and leg discomfort were increased compared with the control trial (P< 0.05) to coincide with reductions in diaphragm contractility during non-potentiated (20.2% ± 15.3%, P<0.05) and potentiated twitches (23.3% ± 12.4%, P<0.05). The extent of diaphragm fatigue observed was also correlated with the inspiratory elastic work of breathing (r^2 = 0.74, P

>0.05) and it was concluded that fatigue of the diaphragm occurs under restricted conditions during submaximal exercise (Tomczak et al., 2011).

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See the following reference for full image:

Tomczak, S. E., Guenette, J. A., Reid, W. D., McKenzie, D. C., & Sheel, A. W. (2011). Diaphragm fatigue after submaximal exercise with chest wall restriction. Med Sci Sports Exerc, 43(3), 416–24.

Figure 1.23 Maximal flow volume loops and tidal breaths at the 10th minute of exercise in both the control (A) and chest wall–restricted (B) condition (Tomczak et al., 2011).

The implications of respiratory muscle fatigue in an occupational setting can impair whole body performance (Brown & McConnell, 2012) and the implications of this have been discussed at length in Section 1.2, however to date there is little information that has sought to investigate the effects of dynamic exercise upon respiratory function during exercise with thoracic loads.

1.4.3.3 ROLE OF CHRONIC LOADING ON LOAD CARRIAGE PERFORMANCE

To date, one study has been conducted using IMT in occupational groups that used 7 professional firefighters and 5 matched civilian volunteers (N=12) who completed an occupational fire test either side of 6 weeks of IMT (Donovan & McConnell, 1999). The fire test was a nine stage, sub-maximal, progressive, test of twenty-three minute duration where all walking stages were performed on a motorised treadmill at a speed of 5.5km·h^{-1} with a

gradient of 6% that was conducted both with and without fire-fighting ensemble. Following IMT, P_{Imax} increased by 25% (P<0.01) which showed an inverse relationship with %Δ post IMT. Although the findings demonstrate a positive effect upon both resting P_{Imax} and ventilatory parameters, the study here failed to employ a control group so the effectiveness of the intervention remains questionable. To date there are no studies to investigate the effects of chronic inspiratory loading on performance in occupational groups that exercise with thoracic loads and any potential benefit has yet to be determined. There is a need for greater knowledge and understanding of the respiratory system limitations to tasks that encompasses thoracic loads that are carried upon the trunk for prolonged durations. This information should include specific detail on respiratory muscle fatigue to determine whether specific interventions that target the trainability of the respiratory muscles are necessary.

1.5 RESEARCH AIMS

To summarise, the respiratory system is tasked with sustaining alveolar ventilation and regulates both blood gas tensions and acid-base balance and although during dynamic exercise this is completed with notable accuracy, it is achieved via regular rhythmic contractions of the respiratory muscles. Sustained contractions of the inspiratory muscles during whole body exercise at high intensities (>85% $\dot{V}O_2$ peak) results in the onset of respiratory muscle fatigue and compromises whole body performance during exercise task, although this is not true of incremental exercise tasks (Romer et al., 2006). The implications during exercise tasks without thoracic loads has outlined a reduction in exercise capacity and that thoracic restriction alone causes diaphragm fatigue (Tomczak et al., 2011), however, the implications for exercise performance with external loads, such as those worn during occupational and recreational tasks, to date remains largely unknown. Alterations in breathing patterns have been observed during loaded tasks due to a volume limitation that is

subsequently imposed upon the thorax which reduces operational lung volumes and alters respiratory muscle efficiency, leading to an increased WOB. It therefore can be postulated that an increased WOB that is sustained for prolonged periods of time and may lead to the development of respiratory muscle fatigue at lower exercise intensities than previously observed within literature. The implications for whole body performance however remain unknown as the effect of thoracic loads on the development of respiratory muscle fatigue during dynamic exercise with thoracic loads has yet to be determined. The use of loading techniques have thus far sought to demonstrate that the fatiguing work of respiratory musculature can be attenuated due to increased respiratory muscle strength and structural adaptions which consequently reduce the work of breathing, perceptual responses, delaying the onset of the metaboreflex and thus increasing whole body performance on tasks. To date however, and due to the lack of knowledge surrounding thoracic load carriage and whole body exercise performance, the implications of IMT techniques have yet to be investigated.

In order to test the hypothesis outlined above, five primary studies were designed. ***Firstly,*** to determine the reliability of a preloaded treadmill time-trial protocol on respiratory muscle strength, pulmonary function and other key physiological responses to exercise that incorporates both submaximal and maximal time trial exercise. There is a need for a protocol that incorporates current military selection tasks that can be used to assess both submaximal and maximal load carriage performance. The protocol must maintain a high level of ecological validity compared with previously used laboratory protocols devised to assess the physiological responses during exercise that closely reflects current operational demands within military settings. ***Secondly,*** using the reliable protocol outlined above to quantify the effects of 25kg thoracic load carriage upon respiratory muscle fatigue, pulmonary function, physiological and perceptual responses during whole body exercise. It is expected here that altered breathing mechanics will induce respiratory muscle fatigue following both

submaximal and maximal exercise and also reduce physical performance when compared to exercise conducted without a thoracic load.

Thirdly, assess if the use of acute inspiratory loading techniques combined with an active warm-up will improve performance on a self-paced, 2.4 km time-trial with 25 kg thoracic load. It is anticipated that a transient increase in respiratory muscle strength will result in improved time-trial performance and attenuate inspiratory muscle fatigue, perceptual and metabolic responses. ***Fourthly,*** identify the effects that chronic loading via the use of six week IMT upon physiological and perceptual responses during 60 min submaximal walking exercise and upon 2.4 km self-paced running time-trial while carrying a 25kg load. It is anticipated here that IMT will increase inspiratory muscle strength; reduce inspiratory muscle fatigue and perceptual responses and in turn, improve time-trial performance. ***Finally***, to evaluate the use of functional IMT methods in attenuating inspiratory muscle and locomotor muscle fatigue that has been previously observed in load carriage activities. It is hypothesised that combining inspiratory muscle training with specific core exercises that target both aspects of the inspiratory muscles dual roles will improve respiratory muscle strength and load carriage performance above that of traditional IMT methods outlined previously, and also attenuate both locomotor and respiratory muscle fatigue.

CHAPTER 2

GENERAL METHODS

2.1 PARTICIPANT PREPARATION

Prior to all research studies, participants were fully briefed and provided with information packs containing a full description of the aims, potential risks and benefits of the research. Following this, participants provided written informed consent, completed a health screen questionnaire and were familiarised with all testing protocols and equipment. The day preceding, and the day of a research trial, participants were instructed not to engage in any strenuous exercise. Each participant completed a 24 h diet record before their first trial and this was repeated prior to subsequent tests. Participants arrived at the laboratory 2 h postprandial during which they were instructed to consume only water having abstained from alcohol and caffeine in the 24 h prior to testing.

2.2 WOODWAY DESMO TREADMILL

All exercise trials were performed on an externally calibrated slat-belt treadmill (Woodway, Desmo, Weil am Rhein; Germany) as shown in Figure 2.1. To ensure that the calibration was sustained, treadmill speed was monitored at 0.5 $km \cdot h^{-1}$ intervals from 6.5 $km \cdot h^{-1}$ to 20 $km \cdot h^{-1}$. Coloured dots were placed on one of the individual slats and the treadmill was started at 6.5 $km \cdot h^{-1}$, which was the intensity used during the fixed intensity phase of the protocol outlined in Section 2.10.3.2.2. Once the treadmill reached target speed, the revolutions were counted over a period of 60 s and multiplied by the length of the belt (1.73 m) to determine distance travelled in a minute ($m \cdot min^{-1}$) and subsequently processed to calculate distance travelled in one hour ($km \cdot h^{-1}$). A 30 s time period was allowed between each incremental stage to account for the known delay that occurs when adjusting the speed. The results (Figure 2.2) demonstrate a near perfect correlation (r = 0.99) with the distance covered and the stated speed on the digital display (mean difference: -0.06 ± 0.08 $km \cdot h^{-1}$, 95% CI for lower LoA: -0.16 – -0.27 $km \cdot h^{-1}$, 95% CI for upper LoA: 0.03 – 0.14 $km \cdot h^{-1}$).

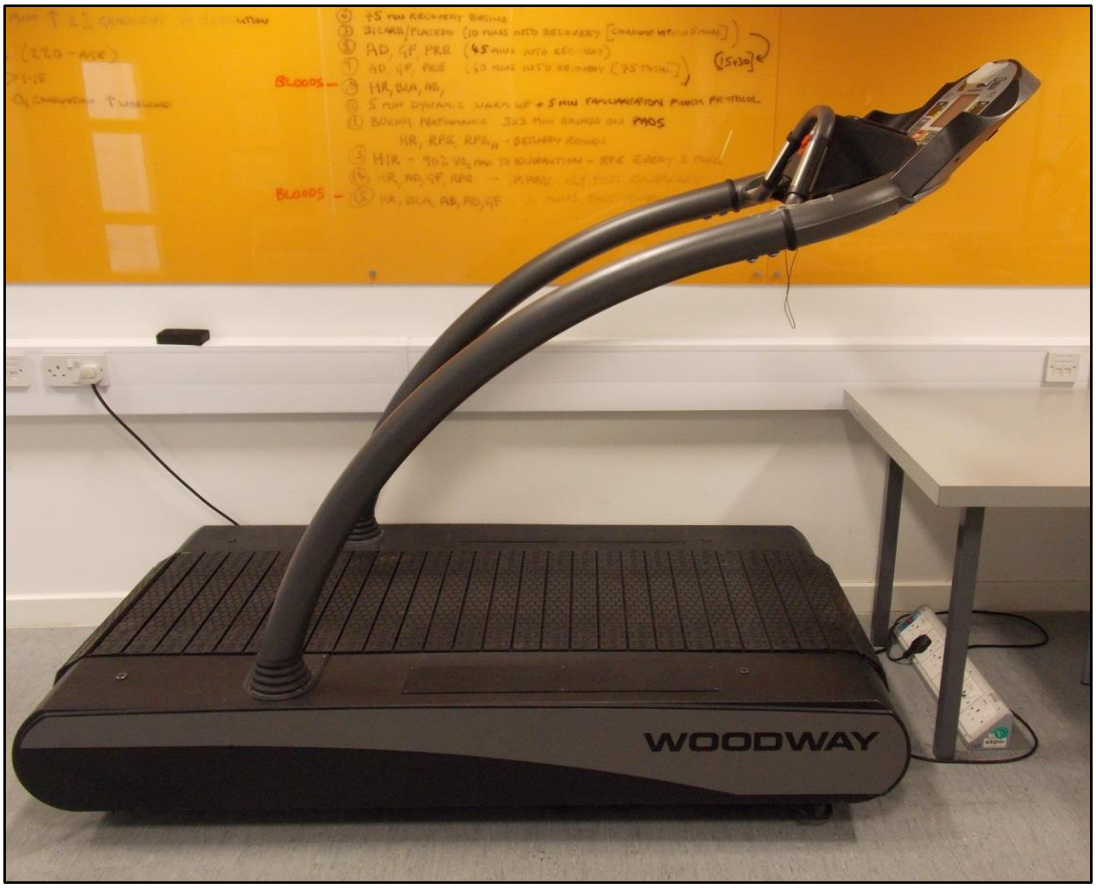

Figure 2.1 Woodway Desmo treadmill used during preliminary, familiarisation and experimental trails for all experimental chapters.

During familiarisation trials, participants were guided through the treadmill controls and digital display; the treadmill remained unaltered with the exception of the time elapsed which was blinded from participants during all trials. The operation of the treadmill was controlled by the researcher, apart from during LC_{TT} (outlined in Section 2.10.3.2.2) which was altered by the participant. The elapsed time was masked from the participant during all trials as shown in Figure 2.2. In alignment with the manufacturer's recommendations and to ensure safety during all aspects of experimental trials, participants were affixed with the built-in safety lanyard in-case of emergency; if this became detached for any reason from its housing on the front panel the treadmill belt was immediately brought to a controlled stop.

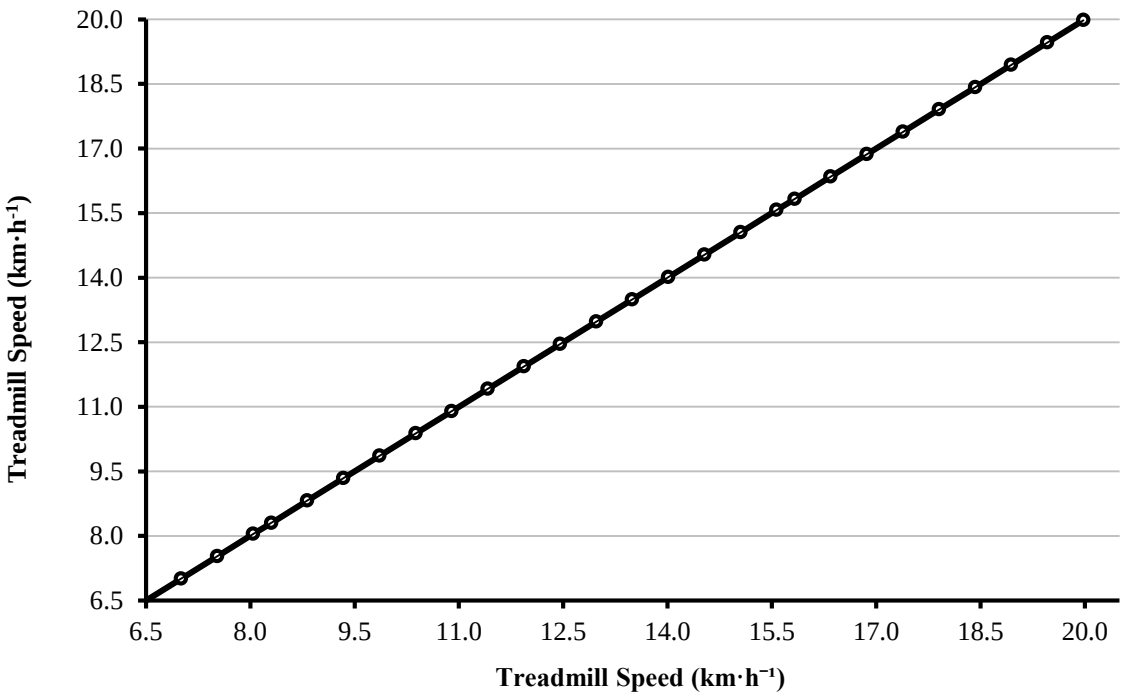

Figure 2.2 Actual vs displayed speed of the Woodway Desmo treadmill used during experimental trials, demonstrating a near perfect correlation (r=0.99).

Figure 2.3 Representation of the treadmill display that was masked to blind participants from the elapsed time during all trials.

2.3 VOLITIONAL MOUTH PRESSURES

A hand-held mouth pressure meter (Micro R.P.M., Micro Medical, Buckinghamshire, UK) was used to assess both maximal inspiratory and maximal expiratory mouth pressures in accordance with previously published guidelines (American Thoracic Society & European Respiratory Society, 2002; McConnell, 2007). Although non-volitional measures are preferred, due to technical limitations and superior between day (i.e., pre to post intervention) reproducibility (Hart et al., 2001; Romer & McConnell, 2004a) volitional manoeuvres were favoured during all experimental chapters. Although the measures used here are highly dependent upon participant motivation, great time and care was taken to ensure full familiarisation prior to experimental trials with the measurement techniques and procedures. Motivation was also provided throughout all efforts; therefore limiting any potential effects of reduced subject motivation and effort and placing confidence in the obtained data. These measures were tested in this thesis for between sessions reliability during the exercise protocol detailed in Section 2.10.3.2.2, demonstrating excellent reliability (See Tables 2.2 and 2.3). This lends support to the findings of Romer and McConnell (2004), who showed that when procedures were conducted correctly and with adequate familiarisation, volitional measures of respiratory function provide reliable and useful data.

The device (Figure 2.4) was externally calibrated and serviced by the manufacturer prior to each experimental chapter to ensure accuracy and precision of measurement. The device consists of a microcomputer unit and two interchangeable pressure valves which serve as separate attachments for the assessment of maximum inspiratory pressure (P_{Imax}) and maximum expiratory pressure (P_{Emax}). Both mouthpiece assemblies incorporate a 1 mm orifice allowing a controlled leak of air, which is important in preventing glottic closure

during both P_{Imax} and P_{Emax} manoeuvres and minimising the contribution of the buccal muscles to generating pressure.

Figure 2.4 Micro RPM used to collect values of maximal inspiratory pressure and maximal expiratory pressure during familiarisation and experimental trails for all experimental chapters.

2.3.1 INSPIRATORY PRESSURE MANOEUVRES

Inspiratory manoeuvres were measured during quasi-static contractions (Mueller manoeuvre) that were performed in an upright standing position during experimental Chapters 3, 4 and 5 and in an upright seated position during experimental Chapter 6. All efforts were initiated from residual volume (RV) and participants were instructed to contract the inspiratory muscles forcefully during each manoeuvre, all efforts were accompanied with motivation and sustained for at least 2 s. Inspiratory efforts were separated by 30 s and

repeated until serial measures were within 10% or 10 cmH_2O of one another with the highest value recorded for analysis (McConnell, 2007).

2.3.2 EXPIRATORY PRESSURE MANOEUVRES

P_{Emax} measures were performed in an upright standing position apart from in experimental Chapter 6 where this was conducted in an upright seated position. P_{Emax} measures were initiated from total lung capacity (TLC) and participants were instructed to exhale forcefully for no longer than 3 s. During expiratory manoeuvres there is a tendency for leakage to occur, which is caused by increased pressure development around the mouthpiece. Participants were instructed here to encircle their lips around the mouthpiece and pinch this in place with the thumb and forefinger (McConnell, 2007). Expiratory efforts were separated by 30 s and repeated until serial measures were within 10% or 10 cmH_2O of one another with the highest value recorded for analysis (McConnell, 2007).

2.4 PULMONARY FUNCTION

Forced dynamic lung volumes and flow were assessed using a handheld device (MicroPlus, Micro Medical, Buckinghamshire, UK) that was externally serviced and calibrated by the manufacturer prior to use in experimental chapters (Figure 2.5). Phototransistors within the device detect movement of the turbine which breaks infra-red beams provided by an LED, twice per rotation when flow is present. The unidirectional turbine measures flow via the number of revolutions of the turbine over a period of time which is proportional to the rate of flow. Each manoeuvre undertaken provides data for: forced vital capacity, defined herein as FVC (L), forced expiratory volume in 1s defined as FEV_1(L) , FEV_1/ FVC (%) and peak expiratory flow defined as PEF ($L \cdot min^{-1}$). The highest values recorded during attempts were reported for analysis and compared with published reference equations (Quanjer et al., 2012).

2.4.1 SPIROMETRY MANOEUVRES

All manoeuvres of dynamic spirometry were assessed in accordance with previously published guidelines (American Thoracic Society & European Respiratory Society, 2002; McConnell, 2007) and conducted in a standing upright position, apart from during the last experimental study where they were performed seated. Measures were initiated from TLC and participants continued until they reached RV: participants completed a minimum of three efforts, separated by 30 s rest.

Figure 2.5 Micro plus hand held peak flow meter used to assess forced dynamic lung volumes during familiarisation and experimental trails for all experimental chapters.

2.5 GAS ANALYSIS

Two methods were used within this thesis to measure pulmonary gas analysis; these techniques were measured at the mouth using both the Douglas bag method in Chapters 3 and 5 and also online breath by breath gas analysis in Chapters 4 and 6. The order in which the studies were conducted saw Douglas Bag techniques used during the first two experimental

studies (Chapters 3 and 5). The breath by breath system used in the last two experimental studies (Chapters 4 and 6) and in favour of Douglas Bag techniques was due to more recent findings from both existing load carriage and IMT research that demonstrated within session changes in gas exchange variables during extended periods of exercise. Therefore the greater requirements and more intricate analyses were required within these chapters.

2.5.1 DOUGLAS BAG METHOD

Samples were collected via a Douglas Bag (Cranlea, Birmingham, UK) which was connected via Falconia tubing to a one way non re-breathable mouthpiece (Hans-Rudolph, 2700 Series, Cranlea, Birmingham, UK). The re-breathing valve contains two valves which permit the air flow in only one direction as demonstrated in (Figure 2.6). During all trials using this method, 60 s samples were collected during the first minute of exercise (baseline) and at minutes 15,30, 45 and during the final minute of exercise during the 60 minute load carriage walk (described in section 2.10.3.2.2). During the self-paced 2.4 km time trial, 60 s samples were collected immediately prior and following the self-paced. All samples were then analysed immediately after collection as described in the below.

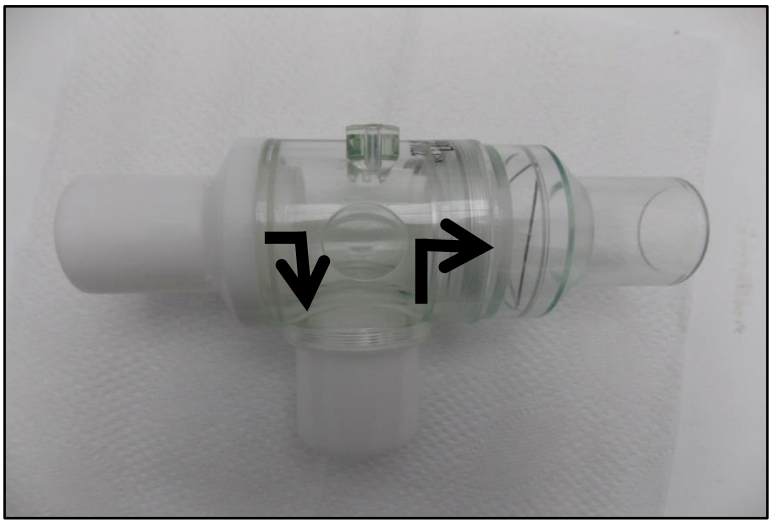

Figure 2.6 One way non re-breathable valve, used to collect Douglas bag samples during Chapters 3 and 5.

2.5.1.1 GAS SAMPLE ANALYSIS

The gas analyser (Hitech Instruments, GIR250, Cranlea, Birmingham, UK) was calibrated prior to each testing session. First, nitrogen (F_IN: 1.0) was first drawn through the device to remove any previous sample residue left within the device, thus allowing the lower limits of both the O_2 and CO_2 cells to be set to zero. Following this, span gasses with known values of O_2 (0.17) and CO_2 (0.05) were used to set the upper limits for both O_2 and CO_2 (Cranlea Disposable Calibration Check Gas, Cranlea, Birmingham, UK). Upon successful calibration, the Douglas bag was connected to the gas analyser via two sample lines (sample in and sample out) which was analysed firstly for the fractional composition (%) of both O_2 and CO_2. Sampling was conducted for 2 min at a flow rate of 2 $l \cdot min^{-1}$ and the concentrations were recorded from the digital display. The contents of the sample were then drawn through the dry gas meter (Harvard Apparatus, Cranlea, Birmingham, UK) to determine both the volume of the sample and the temperature (^oC) within the bag. The barometric pressure (mmHg) of the room was also recorded (Technoline, Cranlea, Birmingham, UK) to allow for appropriate conversion into $\dot{V}_{ESTPD}$. This is necessary as the volume of a gas depends upon the temperature, pressure and the saturation with water molecules, expired gas volume is measured under ambient temperature and pressure, saturated conditions ($\dot{V}_{EATPS}$), because the temperature and pressure of our surroundings is variable, a volume collected under saturated conditions must be standardised by converting to the $\dot{V}_{EATPS}$ to standard temperature and pressure, dry conditions ($\dot{V}_{ESTPD}$). Therefore these values are corrected accordingly using a conversion table (See Appendix 1). These values obtained here are then subject to a series of equations (See Appendix 2) via a Microsoft Excel spreadsheet to determine both O_2 and CO_2 consumption.

2.5.2 CORTEX METALYSER II

Samples of pulmonary gas exchange and ventilation were also measured at the mouth using an online breath by breath analyser (Cortex Biophysik, Metalyser II, Leipzig, Germany) that was operated via a laptop (Helwett Packard, Probook 650, London, UK) during Chapters 4 and 6. After calibration of the device as described below, participants were fitted with a facemask (Hans Rudolph, FCM00201B, Glamsbjerg, Denmark) that covered both the mouth and nose and a skull cap (Hans Rudolph, FCM00101, Glamsbjerg, Denmark) as shown in Figure 2.7. Flow provided during inspiration and expiration was measured by a volume transducer containing a bi-directional turbine (Cortex, Triple V, Cranlea, Birmingham, UK). The level of flow is determined by the presence of phototransistors within the turbine that detect the rate of flow during the breathing cycle. Gas samples are also analysed via the gas line which inserts directly into the turbine. From here gas is drawn into the device and aligned with expired airflow using a software package (MetaSoft Studio version, Cortex, Leipzig, Germany) to provide pulmonary gas exchange measurements expressed at standard temperature, pressure and dry (STPD). Samples were collected throughout the duration of all exercise tasks and are reported at baseline (recorded one minute preceding exercise), post-60 load carriage and post load carriage time trial, both were recorded in the final minute of exercise. The raw values for selected variables collected was exported to Microsoft Excel 2010 and analysed. Subsequently the data provided for experimental chapters 4 and 6 is shown as 30 s averages to minimise possible error in the breath by breath signal from erroneous breaths (e.g. cough and sighs).

Figure 2.7 Hans Rudolph face mask and skull cap assembly used for collecting breath by breath samples during preliminary assessments for all experimental chapters and during experimental trials during experimental Chapters 4 and 6.

The device was calibrated prior to each experimental trial, and the results of these calibrations were logged manually to monitor deviation in the calibration values. If it was suspected that the calibration values were abnormal, or did not meet the pre-set requirements of the software package the device was recalibrated until acceptable values were obtained. Calibration occurs in two parts, firstly flow was calibrated using a 3 litre syringe (Hans Rudolph, 5530, Glamsbjerg, Denmark) and both inspiratory and expiratory factors, defined as the difference between a known value and the actual reading were logged. Secondly, the O_2 and CO_2 sensors were calibrated; to begin the sample line was positioned away from direct contact with direct breathing and draughts and the device was initiated to draw through ambient air for a period of ~2 min, this was followed by the use of span gasses of known concentration (as described in Section 2.5.1.1) which align the sensors to provide expected

values of both O_2 and CO_2. Collection of gasses was continuously monitored throughout preliminary testing (Section 2.10.1) and experimental testing in Chapters 4 and 6. Data points were collected at baseline and 15 minutes intervals throughout the 60 min load carriage task, which were averaged over 60 sec. During the self-paced 2.4 km time trial data points were collected at 400 metre intervals which were averaged over 20 sec periods.

The continuous nature of data collection meant the device was often active for a period ~2.5 hrs. To ensure that the calibration values did not drift during this time frame it was investigated by logging the calibration values on five separate occasions (every 30 min) over a 3 hour period (see Figure 2.8). The data shows consistency and very little deviation in values over time, thus demonstrating precision in both the measuring instrument and the data collected during experimental trials.

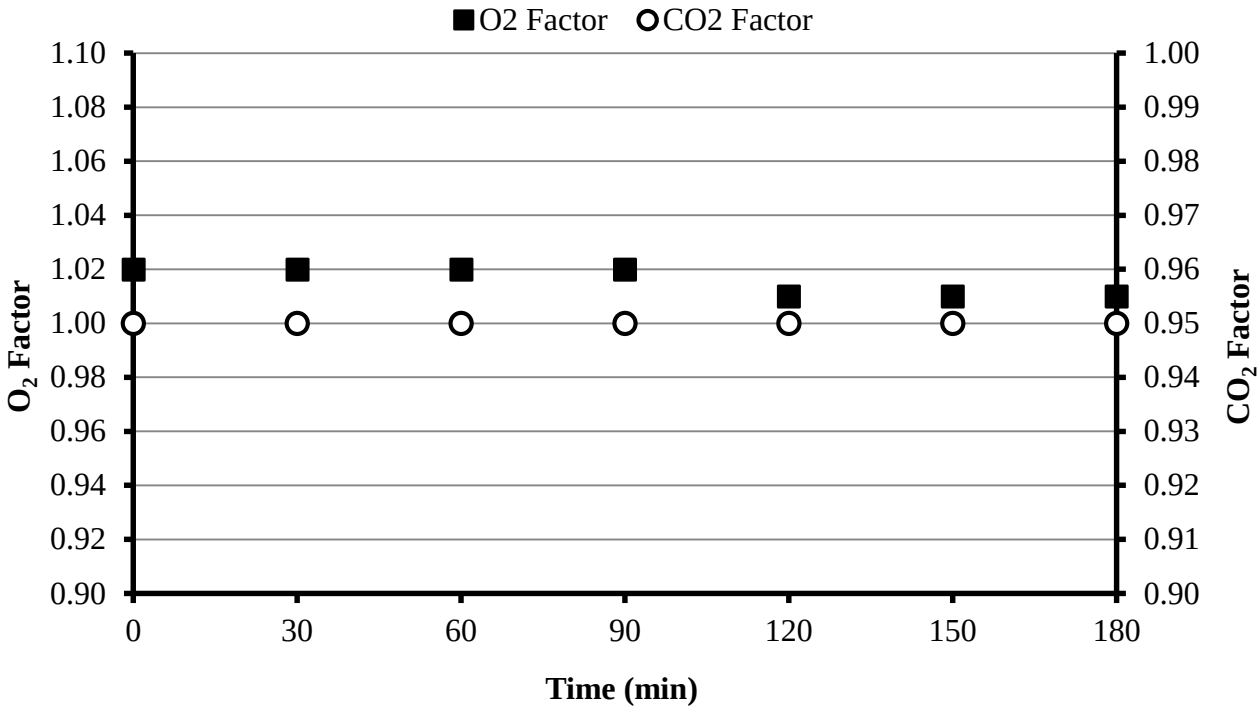

Figure 2.8 Calibration data obtained from the Cortex metalyser over a 180 min period to reflect the duration of the load carriage exercise protocol detailed in Section 2.10.3.2.2.

2.6 BLOOD SAMPLING AND ANALYSIS

2.6.1 SITE PREPARATION AND EXTRACTION

All samples were drawn from the distal phalanx of the index finger; prior to extraction of the sample, the index finger was prepared by cleansing the area with a non-alcoholic wipe (Stereoplast Healthcare, Stereowipe, Manchester, UK) and dried with a paper towel. Following this a single use lancet (Accu-Check, Safe-T-Pro Plus) was used to puncture the site. The first sample of blood was removed as to avoid possible contamination and pressure was applied around the site with the use of the thumb and index finger to draw a second sample for analysis. Once the relevant sample had been collected, the wound was wiped with the paper tissue and the participant was instructed to hold the paper towel firm against the wound until bleeding had ceased. Blood was analysed using two different methods during experimental chapters which are outlined below.

2.6.2 LACTATE PRO BLOOD TEST METER

On successful extraction of the blood from the capillary as outlined above $[lac^-]_B$ concentrations were measured singularly using a Lactate Pro Test Meter (Accu-Check, Safe T-Pro, Birmingham, UK) during Chapters 3 and 5. Blood was drawn into a lactate pro test strip ($[lac^-]_B$; Accu-Check, Safe T-Pro, Birmingham, UK) via capillary action, each sample requires 5µl of arterialised-venous blood. The $[lac^-]_B$ present within the sample is directly proportional to an electrical current measured amperometrically arising from the products of a chemical reaction with potassium, ferricyanide and lactate oxidase (Pyne, Boston, Martin, & Logan, 2000).

Prior to each individual test the device was calibrated; firstly the Lactate Pro is supplied with a Check Strip to confirm that the analyser is operating correctly which is

followed by inserting a calibration strip. The calibration strip is marked with a function number that corresponds to a matching set of test strips and provides a non-quantitative indication of instrument accuracy.

2.6.3 EKF BIOSEN C-LINE CLINIC

Blood sampling in Chapters 4 and 6 was measured using an automated enzymatic method (Biosen, EKF Diagnostics, Barleben, Denmark). Blood samples were collected in 20 µl end-to-end capillary tubes (Biosen, EKF Diagnostics, Barleben, Denmark), which were then placed into a 1 µl micro test tube filled with a glucose/lactate haemolysing solution (Biosen, EKF Diagnostics, Barleben, Denmark) and shaken vigorously for approximately 10 s to ensure dilution.

All samples were measured for ($[Lac^-]_B$ and $[Glu^-]_B$ concentrations and the specific values were then determined immediately using the analyser. The samples are converted enzymatically with the help of the reaction of the immobilised enzyme lactate oxidase and glucose oxidase. The products of this reaction are gluconinc acid, pyruvate and hydrogen peroxide; which are detected using an amperometric electrochemical sensor chip. The product of the aforementioned reaction produces an electrical current on the site of the working electrode which is directly proportional to the original lactate concentration; which is achieved by comparison to the reference electrode site of the micro sensor where no electrical current is present. To prevent contamination of samples, the chip sensor is automatically cleaned with a buffer solution which washes out prevent sample substance prior to analysis. It is noted that from the manual that these measures of $[Lac^-]_B$ and $[Glu^-]_B$ are reliable measures demonstrating a coefficient of variation <1.5% when calibrated using known standard solutions (12mmol/L). Prior to initial use and subsequent 60 min^{-1} intervals, the device was calibrated using a standard solution of known concentration ($[lac^-]$ / [glucose]: 12 $mmol \cdot L^{-1}$).

Previous research has confirmed the intra-sample and between-day reliability of this method (Davison et al. 2000).

2.7 BODY COMPOSITION ASSESSMENT

2.7.1 DUAL X-RAY ABSORPTIOMETRY

All scans were completed by a trained clinical radiographer located onsite at the University of Derby. Briefly, scans were conducted using the criterion method and a comprehensive body composition scanner (Lunar iDXA. GE Healthcare, Buckinghamshire, UK) to quantify bone mass and bone mineral density in addition to fat and lean mass of soft tissue. The principle method here is that the iDXA scanner emits a dual energy beam; one of which is high energy and one of low energy. The lower intensity beam is absorbed by soft tissues (e.g. muscle and fat tissue) and the high intensity beam is absorbed by both bone and soft tissue allowing the software to detect and quantify the absorption of energy within the body and quantify mass, soft tissue mass and also bone mineral density.

2.7.2 BIO ELECTRICAL IMPEDANCE ANALYSIS

Single frequency bioelectrical impedance analysis (BIA) was conducted with the participant resting supine and in the neutral anatomical position, during Chapter 4 only, due to temporary unavailability of the iDXA scanner. After the area of the skin was shaved and cleansed with a non-alcoholic wipe, whole-body resistance was measured using four surface electrodes placed on the right wrist and ankle according to the manufacturer's guidelines (Bodystat 1500, Bodystat Ltd, British Isles). Briefly the scanner (BODYSTAT, BODYSTAT 1500, Isle of Man, UK) passes an electrical current of 400 micro-amps between two sets of leads, each set containing one red (injecting) and one black (measuring) electrode. The leads are attached self-adhesive disposable electrodes that are positioned on the foot and wrist on

the right hand side of the body and are positioned according to the manual. The red electrode on the wrist is proximal to the metacarpophanlgeal joint of the middle finger and the black electrode is positioned just medial to the styloid process of the ulnar. The red electrode on the foot is proximal to the metatarsophalangeal of the second toe and the black electrode is positioned between the lateral and medial malleoli. The device detects the difference in impedance between the body's lean and fat compartments which have very different water contents. The high electrolytic water content present within the lean compartment makes it a good conductor of the current, yielding low impedance compared with the fat compartment which has low levels of water content. The impedance measurement therefore reflects the degree of resistance to the flow of current between the two electrodes. The impedance factor obtained during the test is combined with anthropometric values that are input into the device and subjected to a number of regression equations to provide information on body composition.

A number of considerations exist with this measurement device to ensure reliability. Difficulty remains in being able to measure impedance using electrical measures, the regression equations used to calculate variables are based on numerous assumptions, optimal hydration levels are important to ensure adequate water content within the lean compartment. Finally electrode placement must be as detailed within the manual to further reduce variation. Although the considerations outline suggest potential flaws with this measurement technique, care was taken during preparation to ensure each consideration where possible was attended too prior to measurement. The user guide reports that impedance can be measured between 30 to 1000 Ω with a measurement accuracy of ±3 Ω.

2.8 PHYSIOLOGICAL MEASURES

2.8.1 HEART RATE

Heart rate (HR) was recorded continuously (5 s intervals) during exercise using short-range telemetry (Polar T31, Polar, Kempele, Finland) via a chest strap that was positioned inferior of the pectoral muscles to ensure full contact with the skin. The electrodes positioned on the reverse of the strap were passed under running water prior to placement on the skin to ensure adequate conductivity.

2.8.2 PERCEPTUAL RESPONSES

Ratings of whole body perceived exertion (RPE) were measured using the Borg scale rated from 6 to 20, 6 being "no exertion" and 20 being "maximal exertion" (Borg, 1982). Perceptions of effort were further separated for leg (RPE_{legs}) and breathing ($RPE_{breathing}$) discomfort using a 1-10 visual analogue scale: where 0 = no exertion and 10 = maximal exertion (Verges, Lenherr, et al., 2007).

2.9 STATISTICAL TREATMENT OF DATA

Throughout the thesis, all data are presented as mean ± SD unless stated otherwise. All statistical analyses (including the assessment of normal distribution and homogeneity of variance) were performed using SPSS version 22 for Windows (SPSS, Chicago, Illinois, USA). Homogeneity of variance, i.e. the assumption that the variance of one variable / group was similar at all levels of another variable, was confirmed using Levene's test (Field, 2013). Firstly, a one-way ANOVA was performed upon the deviance scores of the variable(s) to reveal the absolute difference between the mean of the group and each individual variance score. A Levene's statistic greater than 0.05 was used to confirm homogeneity of variance.

For all data included in statistical analyses, normal distribution of the sample was used to indirectly confirm normal distribution expected within the sample population (Field, 2013). Normality was confirmed by interpretation of frequency distribution histogram plots and interpretation of the empirical skewness and kurtosis. Interpretation of the Kolmogorov-Smirnov statistic was also used to objectively interpret whether the distribution of data was significantly different from a comparable normal distribution with an identical mean ± SD. Data was considered normal when the Kolmogorov-Smirnov α-level was greater than 0.05 (Field, 2013).

2.10 SPECIFIC STUDY PROTOCOLS

2.10.1 $\dot{V}O_2$ PEAK ASSESSMENT

A maximal incremental exercise test was performed on a motorised treadmill (Desmo, Woodway, Germany) to determine peak O_2 uptake ($\dot{V}O_2$ peak) during all experimental chapters. Following a 5 min warm-up at 8 $km \cdot h^{-1}$ and 1% gradient, the gradient was subsequently increased to 4% and speed increased by $1km \cdot h^{-1} \cdot min^{-1}$ until the limit of volitional tolerance (Brown, Hughes, & Tong, 2008). Online breath by breath gas analysis (as described in Section 2.5.2) was used to determine $\dot{V}O_2$ peak, which was defined as the highest 30s $\dot{V}O_2$ recorded during the test.

2.10.2 LOAD CARRIAGE PROTOCOL

This thesis designed and tested for reliability a novel load carriage exercise protocol consisting of 60 min walking, 0% gradient and $6.5km \cdot h^{-1}$ (hereon referred to as LC) a 15 min seated rest period and then a 2.4 km self-paced running time trial (hereon referred to as LC_{TT}) where the speed of the treadmill was manually adjusted by the individual to complete the distance in the quickest time possible. As detailed previously and shown in Figure 2.3, the

elapsed time was masked from the participant during all trials. All exercise was performed while carrying a 25 kg thoracic load in a military style backpack (British Army DPM Camo Bergen, 110 L Long Back, Go Army, UK). An absolute load was selected in favour of a relative load to represent a typical load carriage task in an occupational population (Rayson, Holliman, & Belyavin, 2000). A 25 kg load features in part basic training as part of the current entry requirements for serving military recruits (Brown et al., 2010). The walking speed was selected due to its use within previous literature that has investigated occupational requirements during military style load carriage tasks (Blacker et al., 2009; Blacker, Fallowfield, Bilzon, et al., 2013; Blacker, Fallowfield, et al., 2010). The protocol described here was utilised in all experimental chapters, with the exception of Chapter 4, which applied the self-paced time trial protocol only.

During all preliminary exercise sessions, participants were familiarised with the backpack (Figure 2.9) which incorporated shoulder straps and a waist strap, adjusted individually and recorded to the nearest mm for subsequent trials. The mass of the load was evenly distributed within the central compartment of the backpack and worn in accordance with manufacturer's guidelines. Prior to each experimental trial participants conducted a shortened version of the protocol described below to ensure familiarisation, which comprised 20 min walking at 6.5km·h^{-1} carrying and whilst carrying 25 kg load, fifteen minutes rest and 2.4 km self-paced time trial. This was performed to maximise between day reliability (see Section 2.10.3.2.2). For experimental trials and after all resting measures were collected, with participants affixed in the backpack (Blacker, Williams, Fallowfield, Bilzon, & Willems, 2010a; Faghy & Brown, 2014).

Figure 2.9 A) Bergen backpack used throughout all experimental chapters, B) the sand bags used to make 25 kg weight and sheets used to keep the sandbags in place during the trials.

2.10.3 PRELOADED TIME TRIAL TO ASSESS LOAD CARRIAGE PERFORMANCE

2.10.3.1 INTRODUCTION

Studies previously investigating the physiological and performance consequences of load carriage (Section 1.4.2), have typically used protocols that lack ecological validity for occupational load carriage activities. For example laboratory based studies have previously utilised single trials (normally a time trial over a set distance ~2.4 to 20 km) for various durations (up to 30 min) and with varying external loads (15 – 46 kg; (Brown & McConnell, 2012a; Knapik et al., 2012). In a military context, soldiers will often exercise at submaximal (e.g. whilst on patrol) and high intensities (e.g. during engagement; Blacker, Williams, Fallowfield, & Willems, 2011). A laboratory based protocol that closely reflects the operational requirements of occupational groups is challenging to devise as the exercise,

duration, and environmental conditions are difficult to replicate as they vary greatly with each scenario. Nevertheless, there is an apparent need for a laboratory protocol that more closely reflects the physiological characteristics of activities encountered in military situations, such as during training or pre-deployment assessments. The walking speed, duration, rest period and the time-trial distance for the protocol proposed here, were selected to reflect the occupational demands of current British Army training and fitness assessments and was in line with previous recommendations for laboratory-based studies of load carriage (Rayson et al., 2000; Richmond, Rayson, Wilkinson, Carter, & Blacker, 2008). The use of an absolute load mass was also selected in favour of a load relative to body mass to directly reflect current training requirements of occupational groups such as the Armed Forces and Emergency Services (Rayson et al., 2000).

This is the first instance where a protocol of this nature has been devised and the reliability yet to be determined for trials on successive days. Therefore the practical question examined and aim here is whether a laboratory-based protocol that incorporates more realistic occupational demands, i.e., a combination of constant speed low intensity and self-paced high intensity exercise, can demonstrate sufficiently low between session variability and be used to reliably assess LC performance. Accordingly if reliable, this protocol will improve the relevance of future studies within and external to this thesis that investigate load carriage performance, and also provide a useful tool to measure LC specific fitness and responses to possible interventions. It is hypothesised that the protocol detailed will demonstrate very good between trial reliability.

2.10.3.2 METHODS

Following ethics approval from the host university, 8 healthy, non-smoking males with regular experience in recreational load carriage activities, were recruited to/for this study (Table 2.1). Prior to the study participants were fully briefed and completed all procedures outlined in Section 2.1.

Table 2.1 Mean (±SD) for the descriptive characteristics of the participants (n=8)

Descriptive Characteristic	Mean ± SD
Age (Years)	20.9 ± 2.9
Height (m)	1.79 ± 0.45
Body Mass (Kg)	79.40 ± 10.76
Body Fat (%)	21.03 ± 6.53
Lean Body Mass (kg)	59.72 ± 6.03
Fat Mass (kg)	16.66 ± 6.96
Bone mineral density ($g \cdot cm^2$)	1.32 ± 0.13
Bone mineral content (kg)	3.27 ± 0.46
$\dot{V}O_2$ peak ($L \cdot min^{-1}$)	4.30 ± 0.46
$\dot{V}O_2$ peak ($ml \cdot kg^{-1} \cdot min^{-1}$)	53.05 ± 3.21

Values are presented as mean ± SD. $\dot{V}O_2$ peak; maximal oxygen uptake in absolute units and relative to body mass.

2.10.3.2.1 PRELIMINARY TRIALS

Participants were individually briefed on the experimental design before completing three preliminary trials. The first preliminary trial consisted of body composition assessment (Section 2.7.2) followed by an incremental exercise test on a motorised treadmill (Desmo, Woodway, Germany) for the determination of $\dot{V}O_2$ peak (Section 2.10.1). During the second preliminary trial, participants were familiarised and fitted with the 25 kg backpack (Web Tex,

Bedford, UK) and completed 20 min exercise at 0% gradient and 6.5km·h^{-1}. Following 15 min of seated recovery participants then completed a self-paced 2.4 km time-trial, explained fully in the next session. In a third preliminary trial participants completed a full experimental trial identical to the trial detailed briefly below.

2.10.3.2.2 EXPERIMENTAL PROCEDURE

Participants completed the experimental trial on two occasions separated by a minimum of seven days. Participants walked for 60 min, 0% gradient and 6.5km·h^{-1} carrying a 25 kg backpack. Following 15 min of seated recovery participants then completed a 2.4 km LC_{TT}, the speed of the treadmill here was manually adjusted by the individual to complete the distance in the quickest time possible. Mouth pressure measures and pulmonary function were conducted at rest, immediately after LC and LC_{TT} as described in Sections 2.3 and 2.4, respectively. Throughout LC, physiological parameters were measured at 15min intervals, immediately prior to, after 1.2 and 2.4km of LC_{TT}. Heart rate was measured as described in Section 2.8.1, perceptual responses were measured as described in Section 2.8.2 expired pulmonary gases as described in Section 2.5.1, and blood lactate concentrations as described in Section 2.6.1.

2.10.3.2.3 STATISTICAL ANALYSIS

Firstly, mean differences between trials were calculated and paired *t-tests* were used to determine systematic bias between the experimental trials (a-priori $\alpha = 0.05$) using SPSS for Windows (SPSS, Chicago, IL, USA). Secondly, the intra-class correlation coefficient (ICC), coefficient of variation (CV) and Cohen's *d* were calculated as a general indicator of reliability between experimental trials. Following this, absolute limits of agreement were calculated using methods detailed previously (Bland, & Altman, 1986) however, due to the presence of heteroscedasticity in the data (a positive relationship between the absolute

measurement differences and their mean) and in line with previous recommendations (Atkinson & Nevill, 1998), 95% log ratio limits of agreement (LoA) were calculated for all variables using methods detailed previously (Bland, & Altman, 1986). This provides an average dimensionless measurement bias (e.g. general learning effect) and random measurement error (e.g. level of agreement) using Microsoft Excel (Microsoft Corporation, Redmond, WA, USA). In addition, standard error and 95% confidence intervals for the measurement bias and random error components of the limits of agreement were calculated. Finally, the 95% ratio limits of agreement were used to estimate sample sizes required for 5%, 10%, 20%, and 30% effects for a repeated-measures methodological design. Sample size calculations were performed using Microsoft Excel according to published equations (Zar, 2010).

2.10.3.3 RESULTS

2.10.3.3.1 TIME TRIAL PERFORMANCE

Time trial performance was 16.71 ± 1.82 min in trial 1 and 16.37 ± 1.78 min in trial 2 (P>0.05) with a mean difference of 0.34 ± 0.89 min (Figure 2.10). Velocity was fixed during the pre-loaded phase at 1.8 $m \cdot s^{-1}$ but during LC_{TT} increased significantly to 2.42 ± 0.24 $m \cdot s^{-1}$ in trial one and 2.46 ± 0.23 $m \cdot s^{-1}$ in trial 2 with no difference between trials in the magnitude of this increase (P>0.05).

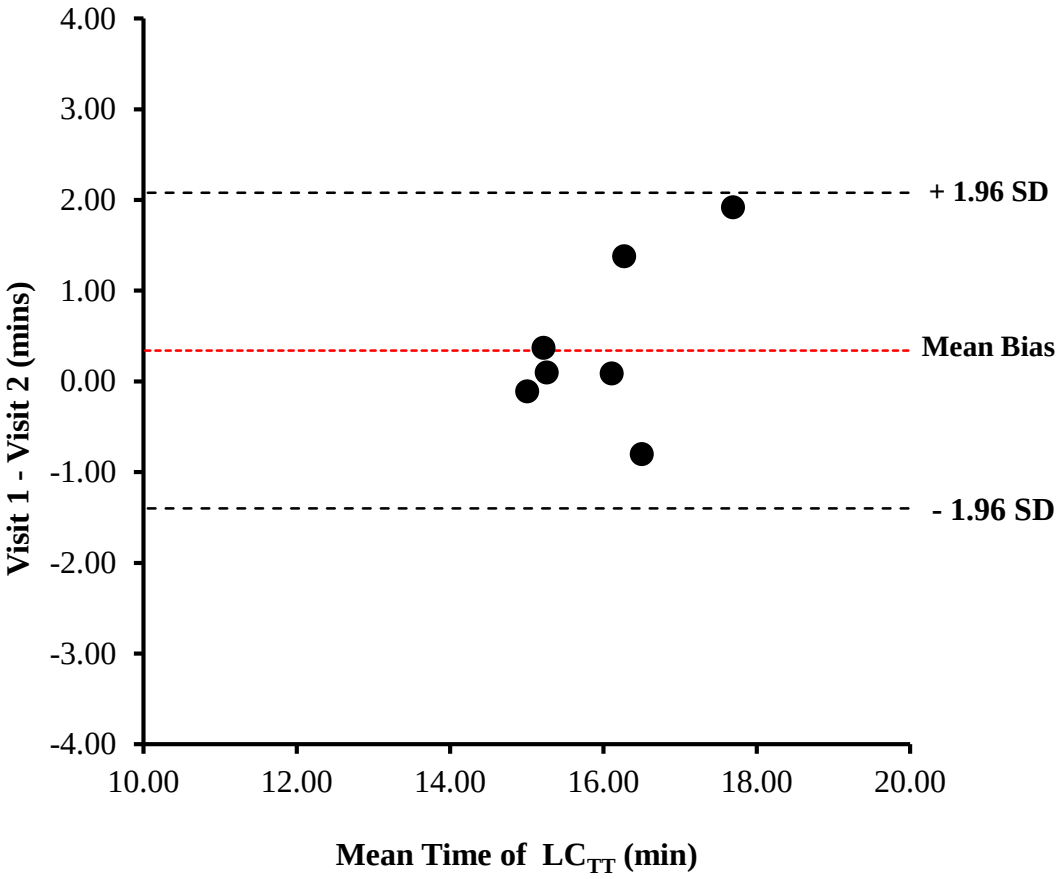

Figure 2.10 Bland-Altman plot for LC_{TT} data achieved during the two experimental trials.

2.10.3.3.2 RESPIRATORY FUNCTION

Relative to baseline P_{Imax} was reduced by 13 ± 11 cmH_2O (11%) and 18 ± 11 cmH_2O (15%) post-LC (P<0.05) and reduced 20 ± 8 cmH_2O (15%) and 21 ± 9 cmH_2O (17%) post LC_{TT} (Baseline to post-LC_{TT}, P<0.05) in trials 1 and 2 respectively. Relative to baseline P_{Emax} was reduced 14 ± 11 cmH_2O and 1 ± 10 cmH_2O in trials 1 and 2 respectively; with further reductions of 1 ± 9 cmH_2O (1%) and 3 ± 11 cmH_2O (3%) in trials 1 and 2 respectively post LC_{TT} (P<0.05). Baseline pulmonary was within normal limits (Quanjer et al., 2012). Relative to baseline; FVC was reduced by 0.35 ± 0.24 L in trial 1 and 0.42 ± 0.26 L in trial 2 (8% and 12%, respectively) post-LC and 0.31 ± 027 L in trial 1 and 0.46 ± 0.31 L in trial 2 (10% and 11%, respectively) post LC_{TT}. FEV_1 was reduced by 0.35 ± 0.24 L in trial 1 and 0.31 ± 0.20 L

in trial 2 (9% and 8%, respectively) post-LC; further similar differences were observed post LC_{TT}, trial 1: 0.31 ± 0.27 L, trial 2: 0.46 ± 0.31 L (8% and 11%, respectively). Increases in FEV_1/FVC were observed relative to baseline during both trials and to a similar magnitude.

2.10.3.3.3 PHYSIOLOGICAL RESPONSES

Physiological responses to both trials are shown in Table 2.2. All transient changes in physiological parameters both post-LC and post LC_{TT} were similar between trials (P>0.05). Baseline HR was 96 ± 19 beats·min^{-1} in trial 1 and 98 ± 21 beats·min^{-1} in trial 2. Post-LC, HR increased by 34 ± 19 beats·min^{-1} and 39 ± 22 beats·min^{-1} in trials 1 and 2 respectively, which was similar between trials (P>0.05). Relative to baseline HR increased post LC_{TT} by 84 ± 28 beats·min^{-1} and 90 ± 24 beats·min^{-1}, respectively (P<0.05).

$\dot{V}O_2$ increased from 1.49 ± 0.18 L·min^{-1} and 1.37 ± 0.38 L·min^{-1} pre-LC by 0.54 ± 0.35 L·min^{-1} and 0.70 ± 0.42 L·min^{-1} post-LC and a further 0.27 ± 0.48 L·min^{-1} and 0.28 ± 0.43 L·min^{-1} post LC_{TT} (P<0.05). $\dot{V}CO_2$ increased from 1.27 ± 0.17 to 1.81 ± 0.35 L·min^{-1} (absolute increase: 0.54 ± 0.35 L·min^{-1}) in trial 1 and from 1.28 ± 0.23 to 1.98 ± 0.31 L·min^{-1} (absolute increase: 0.70 ± 0.42 L·min^{-1}) in trial 2 post-LC (P>0.05). Post LC_{TT} $\dot{V}CO_2$ increased a further 0.27 ± 0.48 L·min^{-1} and 0.57 ± 0.55 L·min^{-1} post LC_{TT} (P>0.05). Baseline V_E was 35.65 ± 4.36 L·min^{-1} in trial 1 and 35.80 ± 6.09 L·min^{-1} in trial 2. Post-LC, VE increased to 49.54 ± 7.42 L·min^{-1} and 52.94 ± 7.77 L·min^{-1} in trials 1 and 2, respectively (P<0.05) and post LC_{TT} increased to 60.52 ± 8.93 L·min^{-1} and 64.08 ± 18.17 L·min^{-1} in trials 1 and 2, respectively (P<0.05).

2.10.3.3.4 RELIABILITY MEASURES

The test-retest and mean difference data for time-trial performance and physiological measurements for LC and LC_{TT} are shown in Table 2.2 and absolute 95% limits of agreement

for the LC_{TT} experimental trials one and two are shown in Figure 2.10. Correlation coefficients of the absolute differences vs. their mean in some variables between trials demonstrated heteroscedasticity therefore, log ratio limits of agreement were calculated for all data sets in addition to ICC, CV and Cohen's *d*. Table 2.3 and 2.4 show the ratio limits of agreement for time trial performance and respiratory function tests post-LC and post-LC_{TT}, respectively and Table 2.5 and 2.6 shows the ratio limits of agreement for physiological and perceptual responses post-LC and post-LC_{TT}, respectively. The estimated sample sizes are displayed in Table 2.7. Overall, for an alpha level of 0.05 and 10% effect, mean sample sizes for all variables were 20 and 22 for LC and $LC_{TT,}$ respectively.

Table 2.2 Absolute test-retest data post 60 minutes of load carriage and 2.4 km time trial for respiratory mouth pressures, pulmonary function, physiological parameters and perceptual responses (n=8).

Variable	Post LC			Post LC_{TT}		
	Mean ± SD1	Mean ± SD2	Difference ± SD	Mean ± SD1	Mean ± SD2	Difference + SD
Exercise Performance						
Time-trial (min)	-	-	-	16.71 ± 1.82	16.37 ± 1.78	0.34 ± 0.89
Respiratory Mouth Pressures						
P_{Imax} (cmH_2O)	115 ± 23	106 ± 20	9 ± 7	109 ± 27	103 ± 19	6 ± 16
P_{Emax} (cmH_2O)	115 ± 21	127 ± 23	12 ± 12	115 ± 28	125 ± 21	10 ± 19
Pulmonary Function						
FEV_1 (L)	4.09 ± 0.79	3.89 ± 0.76	0.20 ± 0.41	3.92 ± 0.74	3.76 ± 0.74	0.17 ± 0.21
FVC (L)	4.96 ± 0.79	4.73 ± 0.77	0.23 ± 0.55	4.78 ± 0.69	4.66 ± 0.85	0.12 ± 0.40
FEV_1/ FVC (%)	82.38 ± 12.98	82.50 ± 12.38	0.13 ± 4.02	81.25 ± 8.41	80.50 ± 10.32	0.75 ± 5.78
PEF ($L \cdot min^{-1}$)	473.88 ± 133.14	474.38 ± 123.35	0.50 ± 29.74	478.00 ± 151.28	457.50 ± 120.95	20.50 ± 132.45
Physiological Parameters						
HR *(beats·min^{-1})*	130 ± 24	137 ± 20	7 ± 11	180 ± 23	188 ± 11	8 ± 13
$[Lac^-]_B$ ($mmol \cdot l^{-1}$)	1.15 ± 0.38	1.19 ± 0.47	0.04 ± 0.49	5.18 ± 2.30	6.08 ± 1.71	0.89 ± 2.39
$\dot{V}_E$ *(L·min^{-1})*	45.54 ± 7.42	52.94 ± 7.77	3.40 ± 9.82	60.52 ± 8.93	64.08 ± 18.17	3.57 ± 18.56
$\dot{V}O_2$ *(L·min^{-1})*	1.88 ± 0.44	1.87 ± 0.45	0.01 ± 0.40	1.21 ± 0.36	1.18 ± 0.40	0.03 ± 0.29
$\dot{V}CO_2$ *(L·min^{-1})*	1.81 ± 0.35	1.98 ± 0.31	0.17 ± 0.42	1.54 ± 0.51	1.85 ± 0.51	0.31 ± 0.36
RER	0.97 ± 0.07	1.08 ± 0.19	0.12 ± 0.15	1.29 ± 0.30	1.63 ± 0.30	0.33 ± 0.28
Perceptual Responses						
RPE (AU)	12 ± 3	12 ± 3	1 ± 2	17 ± 2	18 ± 2	1 ± 1
RPE_{legs} *(AU)*	3 ± 2	3 ± 2	0 ± 1	7 ± 2	7 ± 2	0 ± 2
$RPE_{breathing}$ *(AU)*	3 ± 2	2 ± 2	0 ± 1	7 ± 2	7 ± 1	1 ± 1

Values are presented as mean ± SD. $P_{Imax,}$ maximal inspiratory pressure; $P_{Emax,}$ maximal expiratory pressure; FEV_1, forced expired volume in one second; FVC, Forced vital capacity; PEF, Peak expiratory flow; HR, heart rate; $[lac^-]_B$, Blood lactate; $\dot{V}_{E,}$ minute ventilation; $\dot{V}O_{2,}$ oxygen consumption; $\dot{V}CO_{2,}$ carbon dioxide production; RER, respiratory exchange ratio; RPE, ratings of perceived exertion; $RPE_{legs,}$ RPE specific to the legs; $RPE_{breathing,}$ RPE specific to breathing.

Table 2.3 Ratio limits of agreement (LoA) and reliability data for respiratory and pulmonary function tests conducted post 60 minutes of load carriage at 6.5km·h^{-1}.

Variable	Bias			Random Error						
	Ratio	SE	CI	Ratio	SE	95% CI for lower LoA	95% CI for upper LoA	ICC	CV	Cohen's *d*
P_{Imax} (cmH_2O)	1.08	0.40	0.18 to 1.98	1.11	0.69	-0.053 to 2.59	-0.36 to 2.76	.934	18.98	0.38
P_{Emax} (cmH_2O)	0.91	0.42	-0.05 to 1.86	1.24	0.73	-0.84 to 2.47	-0.53 to 2.78	.860	17.13	0.64
FEV_1 (L)	1.05	0.39	0.16 to 1.94	1.22	0.68	-0.59 to 2.48	-0.25 to 2.82	.918	20.34	0.26
FVC (L)	1.05	0.38	0.18 to 1.91	1.16	0.66	-0.52 to 2.47	-0.28 to 2.71	.646	15.65	0.30
FEV_1/ FVC (%)	1.00	0.37	0.16 to 1.83	1.09	0.64	-0.49 to 2.40	-0.36 to 2.53	.977	15.19	0.21
PEF (L·min^{-1})	0.99	0.38	0.14 to 1.85	1.15	0.66	-0.56 to 2.41	-0.34 to 2.63	.988	26.88	0.20

SE, Standard error; CI, Confidence interval; ICC, Intraclass correlation; CV, Coefficient of Variation; $P_{Imax,}$ maximal inspiratory pressure; $P_{Emax,}$ maximal expiratory pressure; FEV_1, forced expired volume in one second; FVC, Forced vital capacity; PEF, Peak expiratory flow

Table 2.4 Ratio limits of agreement (LoA) and reliability data for respiratory and pulmonary function tests conducted post 2.4 km time trial.

Variable	Bias			Random Error						
	Ratio	SE	CI	Ratio	SE	95% CI for lower L of A	95% CI for upper L of A	ICC	CV	Cohen's *d*
Time (mins)	1.02	0.37	0.18 to 1.86	1.11	0.65	-0.49 to 2.43	-0.33 to 2.59	.850	10.53	0.36
P_{Imax} (cmH_2O)	1.09	0.43	0.07 to 2.02	1.30	0.75	-0.78 to 2.61	-0.33 to 3.05	.860	20.44	0.37
P_{Emax} (cmH_2O)	0.91	0.44	-0.09 to 1.92	1.37	0.77	-0.96 to 2.52	-0.96 to 2.52	.811	19.09	0.64
FEV_1 (L)	1.04	0.37	0.20 to 1.89	1.12	0.65	-0.48 to 2.45	-0.30 to 2.64	.969	18.99	0.36
FVC (L)	1.03	0.39	0.15 to 1.91	1.20	0.67	-0.58 to 2.46	-0.28 to 2.76	.930	15.82	0.29
FEV_1/ FVC (%)	1.01	0.38	0.16 to 1.86	1.07	0.65	-0.53 to 2.43	-0.33 to 2.63	.933	11.08	0.48
PEF ($L \cdot min^{-1}$)	1.03	0.42	0.09 to 1.97	1.38	0.72	-0.76 to 2.51	-0.21 to 3.06	.906	28.32	0.44

SE, Standard error; CI, Confidence interval; ICC, Intraclass correlation; CV, Coefficient of Variation; $P_{Imax,}$ maximal inspiratory pressure; $P_{Emax,}$ maximal expiratory pressure; FEV_1, forced expired volume in one second; FVC, Forced vital capacity; PEF, Peak expiratory flow.

Table 2.5 Ratio limits of agreement (LoA) for physiological and perceptual responses post 60 minutes of load carriage at 6.5km·h^{-1}.

Variable	Bias			Random Error						
	Ratio	SE	CI	Ratio	SE	95% CI for lower L of A	95% CI for upper L of A	ICC	CV	Cohen's *d*
HR *(beats·min^{-1})*	0.94	0.39	0.05 to 1.83	1.23	0.68	-0.69 to 2.39	-0.38 to 2.70	.920	16.49	0.32
$[Lac^-]_B$ (mmol·l^{-1})	0.98	0.51	-0.18 to 2.14	2.07	0.89	-1.33 to 2.68	0.02 to 4.04	.549	29.98	0.88
$\dot{V}_E$ *(L·min^{-1})*	0.94	0.44	-0.05 to 1.92	1.51	0.75	-0.95 to 2.47	-0.30 to 3.12	.311	11.31	0.98
$\dot{V}O_2$ *(L·min^{-1})*	1.01	0.44	0.01 to 2.01	1.56	0.77	-0.94 to 2.54	-0.16 to 3.32	.284	21.12	0.70
$\dot{V}CO_2$ *(L·min^{-1})*	0.91	0.45	-0.11 to 1.93	1.60	0.78	-1.04 to 2.48	-0.30 to 3.22	.769	13.53	1.00
RER	0.90	0.40	0.00 to 1.81	1.27	0.69	-0.77 to 2.36	-0.42 to 2.71	.496	11.48	0.85
RPE (AU)	0.94	0.46	-0.10 to 1.98	1.67	0.80	-1.08 to 2.52	-0.23 to 3.37	.827	23.63	0.60
RPE_{legs} *(AU)*	0.99	0.53	-0.22 to 2.19	2.24	0.92	-1.44 to 2.74	0.12 to 4.30	.903	48.70	0.46
$RPE_{breathing}$ *(AU)*	1.01	0.82	-0.84 to 2.86	5.17	1.42	-2.76 to 3.64	2.04 to 8.44	.946	81.06	0.37

SE, Standard error; CI, Confidence interval; ICC, Intraclass correlation; CV, Coefficient of Variation; HR, heart rate; $[lac^-]_B$, Blood lactate; $\dot{V}_E$, minute ventilation; $\dot{V}O_2$, oxygen consumption; $\dot{V}CO_2$, carbon dioxide production; RER, respiratory exchange ratio; RPE, ratings of perceived exertion; RPE_{legs}, RPE specific to the legs; $RPE_{breathing}$, RPE specific to breathing.

Table 2.6 Ratio limits of agreement (L of A) for physiological and perceptual responses post 2.4 km time trial.

Variable	Bias			Random Error						
	Ratio	SE	CI	Ratio	SE	95% CI for lower L of A	95% CI for upper L of A	ICC	CV	Cohen's *d*
HR *(beats·min^{-1})*	0.95	0.38	0.08 to 1.82	1.18	0.67	-0.63 to 2.38	-0.38 to 2.63	.832	9.21	0.44
$[Lac^-]_B$ (mmol·l^{-1})	0.78	0.57	-0.52 to 2.07	2.57	0.99	-1.76 to 2.72	-0.24 to 4.24	.463	29.07	0.95
$\dot{V}_E$ *(L·min^{-1})*	0.97	0.48	-0.12 to 2.07	1.85	0.84	-1.19 to 2.61	-0.10 to 3.70	.788	17.51	1.10
$\dot{V}O_2$ *(L·min^{-1})*	1.04	0.48	-0.04 to 2.13	1.83	0.83	-1.12 to 2.65	0.02 to 3.79	.295	29.52	0.68
$\dot{V}CO_2$ *(L·min^{-1})*	0.82	0.45	-0.19 to 1.83	1.58	0.77	-1.10 to 2.40	-0.45 to 3.05	.847	28.17	0.75
RER	0.79	0.43	-0.18 to 1.76	1.46	0.74	-1.03 to 2.33	-0.53 to 2.33	.526	18.14	1.03
RPE (AU)	0.94	0.38	0.09 to 1.79	1.14	0.65	-0.60 to 2.36	-0.41 to 2.55	.883	11.70	0.48
RPE_{legs} *(AU)*	1.06	0.49	-0.06 to 2.17	1.92	0.85	-1.17 to 2.69	0.10 to 3.97	.590	21.87	0.81
$RPE_{breathing}$ *(AU)*	0.89	0.43	-0.07 to 1.85	1.44	0.74	-0.93 to 2.41	-0.39 to 2.95	.853	22.59	0.55

SE, Standard error; CI, Confidence interval; ICC, Intraclass correlation; CV, Coefficient of Variation; HR, heart rate; $[lac^-]_B$, Blood lactate; $\dot{V}_E$, minute ventilation; $\dot{V}O_2$, oxygen consumption; $\dot{V}CO_2$, carbon dioxide production; RER, respiratory exchange ratio; RPE, ratings of perceived exertion; RPE_{legs}, RPE specific to the legs; $RPE_{breathing}$, RPE specific to breathing.

Table 2.7 Estimated sample size for different effects in both 60 minutes of load carriage and 2.4 km time trial (n=8).

	60 min Load Carriage Effect Size				2.4 km time trial Effect Size			
Variable	**5%**	**10%**	**20%**	**30%**	**5%**	**10%**	**20%**	**30%**
Time	N/A	N/A	N/A	N/A	11	3	1	0
P_{Imax}	11	3	1	0	98	25	6	3
P_{Emax}	63	16	4	2	150	37	9	4
FEV_1	53	13	3	1	16	4	1	0
FVC	28	7	2	1	44	11	3	1
FEV_1/ FVC	9	2	1	0	21	5	1	1
PEF	25	6	2	1	158	39	10	4
HR	58	14	4	2	35	9	2	1
$[Lac^-]_B$	5	1	0	0	355	89	22	10
RPE	491	123	31	14	21	5	1	1

$P_{Imax,}$ maximal inspiratory pressure; $P_{Emax,}$ maximal expiratory pressure; FEV_1, forced expired volume in one second; FVC, Forced vital capacity; PEF, Peak expiratory flow; HR, heart rate; $[lac^-]_B$, Blood lactate; $\dot{V}_{E,}$ minute ventilation; $\dot{V}O_{2,}$ oxygen consumption; $\dot{V}CO_{2,}$ carbon dioxide production; RER, respiratory exchange ratio; RPE, ratings of perceived exertion.

2.10.3.4 DISCUSSION

This thesis has designed a novel protocol to assess the physiological responses to and performance of load carriage exercise. The data presented above demonstrates a protocol that can be used to assess load carriage performance reliably. The constituent elements of the this closely reflect the intensity and physiological demands of some occupational activities (Rayson et al., 2000). It is also demonstrated here that a range of physiological and perceptual responses can be assessed between trials, with acceptable, between session variability (Atkinson & Nevill, 1998) and is subsequently used in Chapters 3,4,5 and 6. Ratio LoA were also calculated between each trial and the ICC , CV and Cohen's *d*, for which a combination of analyses provides empirical evidence, supporting the inclusion of this performance assessment protocol within this thesis and future studies (Atkinson & Nevill, 1998).

2.10.3.4.1 PERFORMANCE MEASURES

Time trial performance was similar to previous studies where British Infantry recruits were carrying the same mass in a backpack over the same distance however, without a 60 min prior-constant intensity bout (Brown et al., 2007, 2010). In this study mean difference between efforts was 0.34 ± 0.89 min demonstrating excellent agreement (measurement bias: 1.02; random error ×/÷ 1.11). This is supported by narrow confidence intervals as depicted in Tables 2.3, 2.4, 2.5, 2.6 and Figure 2.10. The ICC (0.85) and CV (10.5%) values demonstrated good reliability and Cohen's *d* (0.36) suggests that differences between trial variance was moderate and inconsequential, demonstrating that this protocol is suitable for the use in the analysis of load carriage performance.

2.10.3.4.2 PHYSIOLOGICAL RESPONSES

Between session variability of physiological and perceptual parameters post-LC and LC_{TT} are presented in Tables 2.5 and 2.6 illustrating good agreement and small error ratios. The variation between trials is greater during LC_{TT} than LC, most likely explained by the nature of the time-trial which was self-paced and hence sensitive to changes in the adopted pace. However, the very good agreement of the physiological parameters presented herein demonstrates they can be measured in this protocol reliably between trials and used to quantify the physiological responses to steady state and time trial load carriage activity. Data represented here show similar temporal changes as existing literature investigating the physiological responses to treadmill marching at 6.5 $5km/h^{-1}$ with 25 kg followed by a 2.4 km time trial (Blacker et al., 2009) which are also key determinants of load carriage performance (Brown et al., 2007). Accordingly the protocol presents a useful mode of exercise which reflects the physiological demands of some operational tasks.

2.10.3.4.3 RESPIRATORY PRESSURES AND PULMONARY FUNCTION

Baseline and changes in P_{Imax} and P_{Emax} were similar ($P>0.05$) between trials demonstrating excellent agreement (see Tables 2.3 and 2.4). There was low bias post-LC (P_{Imax} 1.08, P_{Emax} 0.91) and post LC_{TT} (P_{Imax}1.09, P_{Emax} 0.91) and small random error ratios post-LC (x/÷ P_{Imax} 1.11, P_{Emax} 1.24) and post-LC_{TT} (x/÷ P_{Imax} 1.30, P_{Emax} 1.37) (see Tables 2.4 and 2.5). The agreement ratio's for P_{Emax} are larger than anticipated, however the data in Tables 2.3 and 2.4 demonstrate narrow 95% confidence intervals for both the upper and lower bound limits. It is likely that greater variability was witnessed in the post-LC_{TT} measures due to the individual differences in recovery rate post LC, adopted running speeds and time taken to complete the time trial since reductions in respiratory muscle pressures are inversely related to exercise intensity. However, excellent ICC values post-LC (P_{Imax} 0.93, P_{Emax} 0.86)

and post-LC_{TT} (P_{Imax} 0.86, P_{Emax} 0.81); where >0.8 demonstrates a very good level of reliability (Atkinson & Nevill, 1998) which qualifies their use in this protocol.

Reductions in pulmonary function under load carriage conditions have been previously observed (Muza, Latzka, Epstein, & Pandolf, 1989a). This occurs as thoracic restriction modifies the normal breathing mechanics of the exercise hyperpnoea response and consequently heightens the work of breathing (Tomczak et al., 2011) as detailed in Section 1.4.3.2. Measurements of pulmonary function were similar between trials at baseline, post LC and post LC_{TT} (*P>0.05*) and demonstrated excellent agreement with narrow confidence intervals and trial bias (See Tables 2.3 and 2.4). Interestingly, agreement of PEF was poor, probably due to the effort dependence of this parameter during the initial high flow-low volume phase of the manoeuvre. Large variations in PEF occur with only slight changes in the inspiratory muscle recruitment pattern during inspiration by the participants prior to expiration (Romer & McConnell, 2004a). If inspiration from FRC to TLC occurs rapidly and is subsequently followed by a forceful exhalation, a greater PEF will result when compared with a controlled inspiration and longer time spent at TLC due to the elastic recoil properties of the thorax (Tzelepis, Zakynthinos, Vassilakopoulos, Geroulanos, & Roussos, 1997). Neither timing or inspiration strategy were controlled here and, similar to existing research, this is offered as a potential explanation for the heightened variability in PEF post-exercise (Romer & McConnell, 2004a). On this basis studies should control inspiratory flow rate and the time spent at TLC during initial phase of the manoeuvre, when conducting this measurement within a load carriage setting.

2.10.3.4.4 PRACTICAL APPLICATION

Here a novel laboratory based protocol is presented that can be used to reliably assess load carriage performance through a pre-loaded time-trial and a host of physiological markers

that are relevant to the analysis of load carriage performance. This protocol can be adopted in settings where it is necessary to quantify load carriage performance; prior to and following a training or pre-exercise priming intervention where load carriage performance is the dependant variable and importantly prior to an operational deployment where load carriage is considered to be a critical role-related task. Within physical selection tests, armed forces organisations do not currently employ a loaded time trial, rather, they employ unloaded running (typically 2.4 – 3.2 km distance). However, this is known to be a very poor predictor of occupational (i.e., load carriage) performance (Bilzon, Allsopp, & Tipton, 2001). The omission of a loaded time trial is likely to mitigate associated injury risk although our findings suggests that including this test would allow for comprehensive analysis to reliably capture changes in military specific fitness.

It is impossible to devise a laboratory-based protocol which precisely reflects operational requirements since the load, intensity, duration, terrain and environment vary with each deployment or training scenario. However, the protocol presented here is similar to current British Army assessments used in pre-deployment training and fitness assessments. For example, the 2.4 km loaded time trial has been used to determine the physical and physiological responses to acute changes in British Army infantry training programmes (Brown et al., 2007, 2010). In addition, this protocol that is similar to the Infantry Basic Combat Fitness Test where soldiers complete an eight mile course, carrying a load or 25 kg at 15 min·mile^{-1} (6.4 km·h^{-1}) and the Advanced Combat Fitness Test 1 whereby following an 800 m warm up at 15 min·mile^{-1}, participants perform a 2.4 km time trial carrying a 20 kg load (The British Army, 2014). In addition, as shown by the sample size calculations (Table 2.7), controlled trials can be performed effectively, typically with a sample of 20 and 22 for the LC and LC_{TT} trials respectively during a repeated measures design offering a large effect size ($\geq$10%).

2.10.3.5 CONCLUSION

The results from this methodological study suggest that the variability in parameters during LC and LC_{TT} are negligible and the presented load carriage protocol provides a reliable measure for assessing the performance and physiological effects of bearing an external load upon the thorax. This protocol also contains improved ecological validity on submaximal and high intensity load carriage activities compared with previous measures which is the first protocol that has attempted to address the reliability of pre-loaded time-trial performance. The findings here are important as they underpin the experimental design in Chapters 3, 4, 5 and 6; whilst also providing a reliable protocol that can be used within future research design.

CHAPTER 3

THORACIC LOAD CARRIAGE-INDUCED

RESPIRATORY MUSCLE FATIGUE

Published in the European Journal of Applied Physiology – 2014

Winner of the Postgraduate Oral Presentation of the Year award at the BASES Student conference – Cardiff Metropolitan University 2013

3.1 INTRODUCTION

Load carriage is defined as locomotion while bearing a mass upon the torso supported by shoulder straps and/or a hip belt (i.e., a backpack) (Knapik, Harman, & Reynolds, 1996) and it is often essential to integrate wearing a backpack with recreational activities such as hiking and in some occupational settings such as the military and emergency services (Birrell & Haslam, 2010). As discussed in Section 1.4.1, the backpack remains one of the most economical modes to carry the necessary consumables and equipment for a given task (Bastien, Willems, Schepens, & Heglund, 2005). The mass of a backpack in some occupational settings can exceed 75 kg (Brown & McConnell, 2012) and previous studies utilising far lighter loads (25 kg) have demonstrated impaired force generating capacity of locomotor muscles (Blacker, Williams, Fallowfield, Bilzon, & Willems, 2010); however at present, there is a dearth of literature quantifying the effects upon the respiratory musculature. Load carriage presents a unique challenge to the respiratory system by combining chest wall restriction and loading, with recent evidence demonstrating impaired pulmonary function and breathing mechanics during exercise (Tomczak, Guenette, Reid, McKenzie, & Sheel, 2011).

During normal unloaded breathing, initial increases in tidal volume occur through a decrease in end-expiratory lung volume (EELV) which serves to optimise diaphragm function. However, the external restrictive constraint and mass of the backpack further reduces EELV and impairs the normal increase in end-inspiratory lung volume (EILV). This change in breathing mechanics increases the energy cost of breathing (Dominelli et al., 2012) and reduces the efficiency of the respiratory muscles as they work outside of their optimal length-tension relationship (Brown & McConnell, 2012). Notably, studies which have mimicked this inspiratory volume limitation (but not the mass of the load) through chest wall restriction using inelastic strapping demonstrate significant diaphragm fatigue (Tomczak et al., 2011). Recent indirect assessment of the accessory musculature which are tasked with

increasing thoracic volume, demonstrated a reduction in the mean power frequency of the external intercostals and the sternocleidomastoid with 15 kg load, the authors suggested this is illustrative of impaired respiratory muscle function (Nadiv et al., 2012). The effects of load carriage upon the pressure generating capacity of the respiratory muscles is however yet to be determined. Therefore the aim of this study was to examine the effects of 25kg thoracic load carriage upon respiratory muscle fatigue, pulmonary function, physiological and perceptual responses during whole body exercise (constant load and time trial performance).

3.2 METHODS

Following ethics approval from the host University, 19 healthy, non-smoking and physically active males (Table 3.1) provided written informed consent to participate in the study. Prior to the study, participants were fully briefed and completed all procedures outlined in Section 2.1. Following this, participants each visited the laboratory on 4 separate occasions separated by a minimum of 7 days' rest where they conducted a series of preliminary assessments, full familiarisation and an experimental trial both with and without a 25 kg external load.

3.2.1 PRELIMINARY TRIALS

Participants completed two preliminary trials: during the first preliminary trial body composition was assessed using dual energy x-ray absorptiometry (Lunar iDXA, GE Healthcare, Hertfordshire, UK) as outlined in Section 2.7.1. Participants then performed a maximal incremental exercise test whilst running on a motorised treadmill (Desmo, Woodway, Germany) to determine $\dot{V}O_2$ peak as described in Section 2.10.1. During the second preliminary visit participants were familiarised with all testing equipment and protocols and completed baseline pulmonary function (Section 2.4.1) and maximal inspiratory (P_{Imax}) and expiratory pressure (P_{Emax}) measurements (Section 2.3). Following this,

participants were fitted and familiarised with the 25 kg backpack (Web Tex, Bedford, UK) where they completed a second round of pulmonary function, P_{Imax} and P_{Emax} tests to assess if thoracic loading reduced pulmonary function and respiratory muscle strength. Following all baseline measures participants completed a shortened version of the load carriage protocol as that consisted of 20 min treadmill marching at 6.5 km·h^{-1} which was followed by 15 mins seated rest and 2.4 km self-paced time trial.

Table 3.1 Mean ±SD for the descriptive characteristics of the participants (*n*=19).

Variables	
Age (years)	24.7 ± 7.5
Body Mass (kg)	83.3 ± 8.9
Height (m)	1.8 ± 0.6
Body Fat (%)	24.4 ± 5.8
Lean body mass (kg)	58.0 ± 4.1
Fat mass (kg)	18.3 ± 6.3
Bone mineral density (g/cm$_2$)	1.3 ± 0.2
Bone mineral content (kg)	3.2 ± 0.6
$\dot{V}O_2$ peak (L·min^{-1})	4.3 ± 0.6
$\dot{V}O_2$ peak (ml·kg^{-1}·min^{-1})	50.9 ± 5.4

Values are presented as mean ± SD. $\dot{V}O_2$ peak; maximal oxygen uptake in absolute units and relative to body mass.

3.2.2 EXPERIMENTAL TRIAL WITH LOAD CARRIAGE

Participants completed two experimental trials one whilst wearing a 25 kg load contained within a backpack (LC) and an identical trial without the load (CON), the order of trials was randomised for each participant and separated by a minimum of 7 days. Following baseline measurements of P_{Imax}, P_{Emax}, and pulmonary function that were conducted without the additional load; participants then completed 60 min walking, 0% gradient and 6.5km·h^{-1}

(LC), which was followed by a 15 min seated rest period and finally a 2.4 km self-paced running time trial (LC_{TT}). Physiological variables including blood lactate, heart rate, perceptual responses and expired air samples (collected using the Douglas bag method) were collected within the first minute of exercise and at minute 15, 30 and 45, values were also collected immediately prior and following LC_{TT}. To confirm if any reductions in mouth pressure measures were the result of the exercise trial and not due to the imposed chest wall loading and restriction caused by the presence of the backpack; a small sub sample of participants (n=4) stood stationary on the treadmill in a separate trial whilst wearing the 25 kg backpack for the exact duration of the exercise protocol (75 mins).

3.2.3 EXPERIMENTAL TRIAL: WITHOUT LOAD CARRIAGE

The control trial (hereon referred to as CON) was identical to load carriage described above, but performed without wearing the backpack.

3.2.4 STATISTICAL ANALYSIS

Changes in dependent variables over time throughout the experimental trials were assessed using a one-way or two-way repeated measure ANOVA with Bonferroni post-hoc analysis. Interactions were defined for “trial” (LC vs. CON) and “time” (baseline vs. post-60 min vs. post-time trial). Paired samples t-test was used to determine differences between selected variables at specific time points. A priori α was set at 0.05 and all results are presented as mean ± SD. Statistical analysis was performed using SPSS for Windows (SPSS, Chicago, IL, USA).

3.3 RESULTS

3.3.1 RESPIRATORY MUSCLE PRESSURES

Baseline P_{Imax} conducted both with and without the external load were not different during preliminary assessments (P_{Imax} 127 ± 2 cmH_2O and 121 ± 6 cmH_2O, $P>0.05$). P_{Imax} and P_{Emax} did not change during the stationary trial completed by a small (n=4) subsample; P_{Imax} pre 167 ± 19 cmH_2O vs post 167 ± 21 cmH_2O, ($P> 0.05$) and P_{Emax} pre 126 ± 3 cmH_2O vs post 120 ± 5 cmH_2O, ($P> 0.05$).

During experimental trials baseline and changes over time in P_{Imax} and P_{Emax} for LC and CON trials are shown in Figure 3.1 and 3.2 respectively. Baseline P_{Imax} and P_{Emax} were unchanged between trials. Relative to baseline, P_{Imax} was reduced 11% post- LC (pre-vs post: 141 ± 30 vs. 124 ± 29 cmH_2O; $P<0.001$) and a further 5% post LC_{TT} (118 ± 25 cmH_2O; $P<0.001$). Between experimental trials, P_{Imax} was significantly lower post-LC ($P<0.01$) and post LC_{TT} ($P<0.01$) compared to CON and CON_{TT}, respectively (trial x time interaction effect post-LC and post- LC_{TT}, $P<0.001$). P_{Emax} was reduced 13% following LC (pre-vs post: 158 ± 37 vs. 139 ± 34 cmH_2O; $P<0.001$) and a further 6% post-LC_{TT} (130 ± 31 cmH_2O; $P=0.01$) (trial x time interaction effect post-LC and post- LC_{TT}, *$P<0.001$*). Following CON P_{Imax} ($P=0.456$) and P_{Emax} ($P>0.05$) were unchanged relative to baseline. Conversely, following CON_{TT} P_{Imax} (6%; $P>0.05$) and P_{Emax} (10%; $P<0.01$) were reduced relative to post-CON.

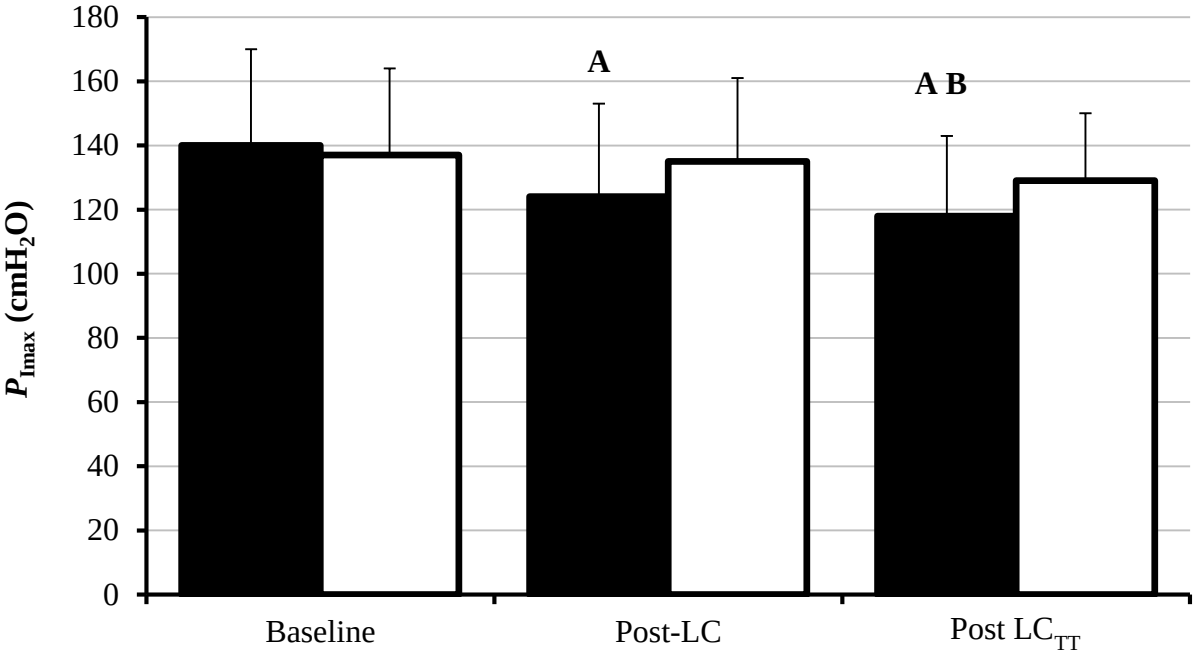

Figure 3.1 P_{Imax} measured at various time points during the load carriage protocol, the black bars represent the loaded trial and the white bars represent the unloaded trial. A, different from baseline (P< 0.05); B, different from post load carriage (P< 0.05).

3.3.2 PULMONARY FUNCTION

Baseline pulmonary function values were different when completed whilst being performed when wearing the load and in an unloaded condition. FEV_1 (without load 4.10 L vs with load 3.81 L P<0.05), FVC (without load 5.12 L vs with load 4.85 L P<0.05) and PEF (without load 492 l·min^{-1} vs with load 455 l·min^{-1}, P<0.05) however FEV_1/FVC remained unchanged (without load 81% vs with load 79%, P>0.05). Baseline pulmonary function was similar between trials (Table 3.2) during CON and reduced at baseline for the load carriage trial (FVC and FEV_1 by 6 ± 13% and 3 ± 9%, respectively). Relative to baseline, FVC was reduced by 2 ± 11% following the loaded trial and 5 ± 6% following LC_{TT}, where FVC was unchanged following CON and reduced by 2 ± 7% following CON_{TT} (P=0.03). Relative to baseline there was a 7 ± 23% reduction in FEV_1 post-LC (P<0.05) which remained unchanged post-LC_{TT} and FEV_1 was unchanged throughout CON and CON_{TT}. PEF remained unchanged

over time in both trials. Increases in FEV_1/FVC relative to baseline were observed during LC and CON and remained unchanged post- CON_{TT} and LC_{TT}.

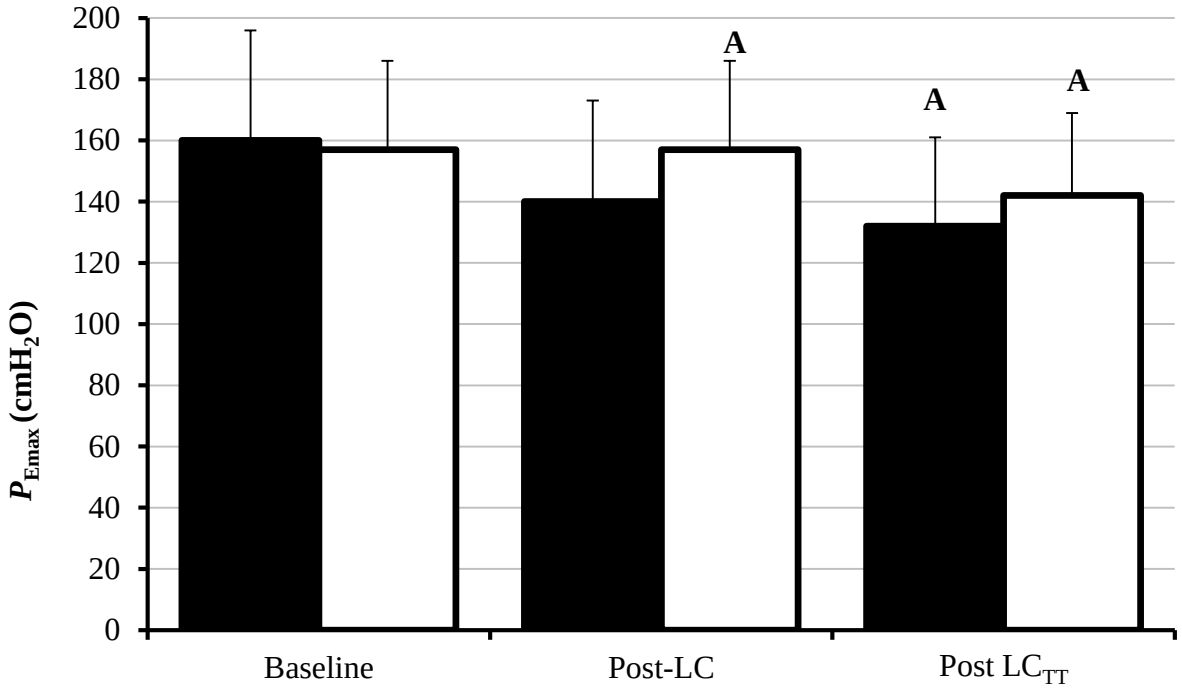

Figure 3.2 P_{Emax} measured at various time points during the load carriage protocol, the black bars represent the loaded trial and the white bars represent the unloaded trial. A, different from baseline ($P< 0.05$).

3.3.3 PULMONARY GAS EXCHANGE AND CARDIOVASCULAR RESPONSES

Changes over time in pulmonary gas exchange and cardiovascular responses for both trials are shown in Table 3.3. With the exception of RER, gas exchange variables: $\dot{V}_{E,}$ $\dot{V}O_2$ *and* $\dot{V}CO_2$ were greater than baseline post-LC and post-CON. Responses were further increased post-LC_{TT} in both conditions ($P<0.05$) with the greatest increase following CON_{TT} ($P<0.05$). HR was also greater than baseline following LC and CON ($P<0.05$). The increase in HR post-LC (absolute increase 46 ± 17 beats·min^{-1}) was greater than post-CON (absolute increase 19 ± 13 beats·min^{-1}, $P<0.05$) and was further increased post-time trial (absolute increase LC_{TT}: 43 ± 18 beats·min^{-1}; CON_{TT}: 76 ± 11 beats·min^{-1}; $P<0.05$).

Table 3.2 Baseline pulmonary function values in both conditions.

	Control (CON)	Load Carriage (LC)
FVC (L)	5.0 ± 0.6	4.7 ± 0.7
FEV_1 (L)	4.0 ± 0.5	3.9 ± 0.5
FEV_1/ FVC (%)	79.1 ± 11.1	81.6 ± 8.7
PEF ($L \cdot min^{-1}$)	525 ± 80	523 ± 96

Values are presented as mean ± SD. FVC, forced vital capacity, FEV1, forced expired volume in one second, PEF, peak expiratory flow.

3.3.4 PHYSIOLOGICAL AND PERCEPTUAL RESPONSES

Changes in the metabolic responses for both experimental trials are shown in Table 3.3. The metabolic cost was 21 ± 2% greater during load carriage (40.2 ± 3.2 $KJ \cdot min^{-1}$) relative to CON (31.8 ± 3.4 $KJ \cdot min^{-1}$, *P<0.01*). Conversely, the metabolic cost in CON_{TT} was 34 ± 8% greater (109.4 ± 26.2 $KJ \cdot min^{-1}$) than LC_{TT} (72.4 ± 15.4 $KJ \cdot min^{-1}$, *P<0.01*). $[glucose]_B$ was similar at baseline between trials and remained unchanged over time. $[lac^-]_B$ decreased following LC (absolute decrease 0.4 ± 1.1 $mmol \cdot l^{-1}$, *P*>0.05) and CON (0.4 ± 0.5 $mmol \cdot l^{-1}$, *P*>0.05) and subsequently increased post-time trial in both conditions with the greatest response observed post-CON_{TT} (absolute increase LC_{TT}: 7.2 ± 3.9 $mmol \cdot l^{-1}$; CON_{TT}: 9.4 ± 2.7 $mmol \cdot l^{-1}$, *P*>0.05). Changes in the perceptual responses for both experimental trials are shown in Table 3.3. All perceptual responses were similar at baseline between trials, but were greater post-LC relative to post-CON (interaction effect: trial x time, *P*<0.01). Responses were further increased (*P*<0.05) following the time trial in both conditions (*P*<0.05) but the absolute increase was not different between trials *P*<0.05).

3.3.5 TIME-TRIAL PERFORMANCE

Group mean changes in 2.4 km time trial performance are shown in Figure 3.3. Time to completion was 30 ± 5% slower in LC_{TT} (15.9 ± 1.9 min) compared to CON_{TT} (11.1 ± 1.6 min; P<0.01). The average speed during LC_{TT} and CON_{TT} was 9.2 ± 1.1 km.h^{-1} and 13.2 ±1.8 km.h^{-1}, respectively (P<0.01).

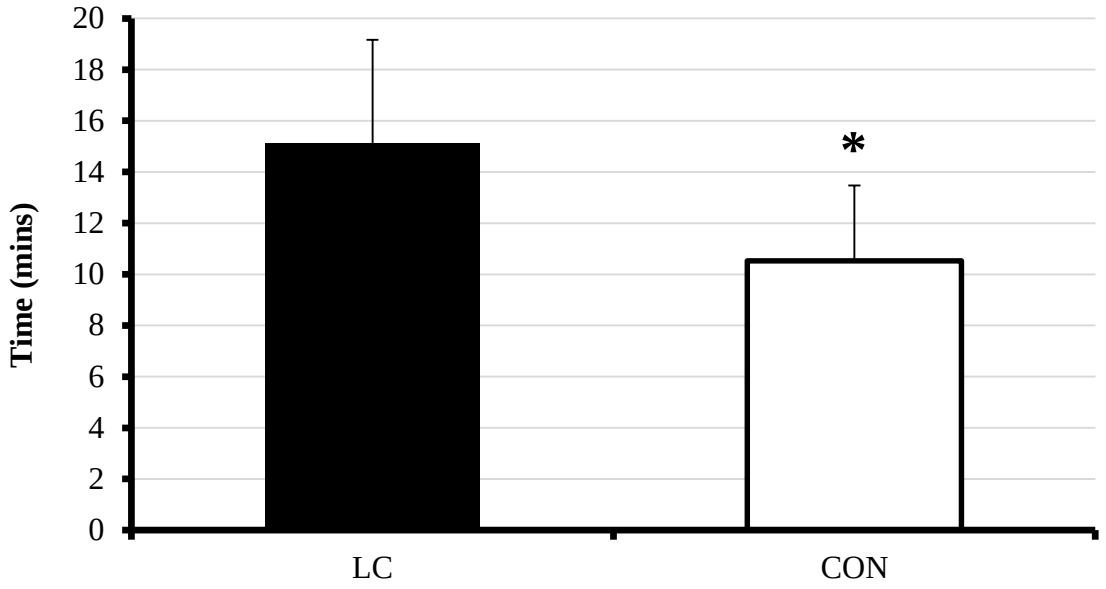

Figure 3.3 LC_{TT} performance in the loaded and unloaded conditions. *, different between trials (P< 0.05).

Table 3.3. Physiological and perceptual responses prior to and following experimental trials.

	Control (CON)			Load Carriage (LC)		
	Pre-CON	**Post-CON**	**Post-CON$_{TT}$**	**Pre-LC**	**Post-LC**	**Post-LC$_{TT}$**
$\dot{V}_E$ (L·min^{-1})	31.0 ± 8.1*	36.3 ± 7.0*^{A}	84.6 ± 20.5*^{AB}	39.9 ± 7.5	48.3 ± 8.0^{A}	61.0 ± 22.0^{A}
$\dot{V}O_2$ (L·min^{-1})	1.3 ± 0.3*	1.4 ± 0.2*	1.7 ± 0.3*^{AB}	1.5 ± 0.3	1.7 ± 0.2	1.3 ± 0.6^{B}
$\dot{V}CO_2$ (L·min^{-1})	1.0 ± 0.3*	1.3 ± 0.31*^{A}	2.6 ± 0.7*^{AB}	1.4 ± 0.2	1.7 ± 0.2 ^{A}	1.7 ± 0.6
RER	0.8 ± 0.1*	0.9 ± 0.1^{A}	1.2 ± 0.4^{AB}	0.9 ± 0.1	1.0 ± 0.2 ^{A}	1.3 ± 0.6^{AB}
$[lac^-]_B$ (mmol·l^{-1})	1.4 ± 0.6*	1.0 ± 0.3*^{A}	10.4 ± 3.0*^{AB}	1.9 ± 0.8	1.5 ± 0.8	8.6 ± 3.5^{AB}
$[Glucose]_B$ (mmol·l^{-1})	4.3 ± 1.3	4.3 ± 0.8	5.4 ± 1.5	4.7 ± 0.8	4.4 ± 0.7	5.3 ± 0.9
HR (beats·min^{-1})	92 ± 15	111 ± 10*^{A}	187 ± 13^{AB}	99 ± 14	144 ± 19^{A}	188 ± 13^{AB}
RPE (AU)	6 ± 0	8 ± 1^{A}	15 ± 4^{AB}	7 ± 1	12 ± 3^{A}	16 ± 2^{AB}
RPE_{legs} (AU)	0 ± 1	1 ± 1^{A}	6 ± 2^{AB}	0 ± 0	3 ± 2^{A}	7 ± 2^{AB}
$RPE_{breathing}$ (AU)	0 ± 0	1 ± 1^{A}	6 ± 2^{AB}	0 ± 0	3 ± 2^{A}	7 ± 2^{AB}

Values are presented as mean ± SD. Values are presented as mean ± SD. $\dot{V}_{E,}$ minute ventilation, $\dot{V}O_{2,}$ oxygen consumption, $\dot{V}CO_{2,}$ carbon dioxide production, RER, respiratory exchange ratio, $[Glucose]_B$, Blood glucose, $[lac^-]_B$, Blood lactate, HR, heart rate; RPE, ratings of perceived exertion, $RPE_{legs,}$ RPE specific to the legs, $RPE_{breathing,}$ RPE specific to breathing. ^{A} P<0.05 different to baseline; ^{B} P<0.05 different to Post;*P<0.05 different between trials.

3.4 DISCUSSION

The purpose of this study was to investigate the effects of 25 kg load carriage during whole body walking exercise upon respiratory muscle fatigue, pulmonary function, physiological and perceptual variables and a 2.4 km time trial performance. The novel finding of this study was that 60 min load carriage induced significant inspiratory and expiratory muscle fatigue which was further exacerbated by the 2.4 km time trial.

3.4.1 RESPIRATORY MUSCLE FATIGUE

For the first time it has been demonstrated that a significant reduction in the voluntary pressure generating capacity of the respiratory muscles following 60 min walking and a self-paced 2.4 km time trial with 25 kg load carriage. An 11% reduction in P_{Imax} and a 13% reduction in P_{Emax} (P<0.05) was observed following load carriage (no change in CON) which was exacerbated by a further 5 and 6% respectively, post-LC_{TT} (P<0.05). Despite these observations, evidence from whole body exercise suggests that respiratory muscle fatigue only occurs following constant power, high intensity exercise to exhaustion (>85% $\dot{V}O_2$ peak), time trial events (Johnson, Sharpe, & Brown, 2007; Leddy et al., 2007; Romer et al., 2002a; Volianitis, McConnell, Koutedakis, McNaughton, et al., 2001); or prolonged sub-maximal (marathon and ultramarathon) exercise (Ross, Middleton, Shave, George, & McConnell, 2008; Wüthrich et al., 2015). Previous occupational research has observed reductions in P_{Imax} and P_{Emax} (10-12%) following sub-maximal and intense treadmill exercise while wearing self-contained breathing apparatus and fire-fighter apparel (Butcher, Jones, Mayne, Hartley, & Petersen, 2007). However this occupational model fails to distinguish between the effects of the load mass secured to the thorax (gas cylinders) and the positive pressure provided by the face mask since the latter accounts for the majority of the increased work of breathing during exercise (Eves et al., 2005). The findings of this study are however

in agreement with previous research which mimicked the restrictive characteristics of a backpack (but not the load mass) and observed reductions in twitch trans-diaphragmatic pressure during 10 min cycling exercise at 40% maximal workload (Tomczak, Guenette, Reid, McKenzie, & Sheel, 2011), which presumably would be exacerbated with the addition of chest wall loading as demonstrated here. In addition, a reduction in the mean power frequency of both the external intercostals and the sternocleidomastoid was demonstrated following 25 kg load carriage is suggestive of impaired respiratory muscle function (Nadiv et al., 2012). However, the validity of these measures has been questioned, such that it correlates poorly with mechanical indices of skeletal muscle fatigue (Sheel & Romer, 2012). Thus, this study extends previous findings by demonstrating that load carriage reduces the threshold by which whole body exercise induces respiratory muscle fatigue to include sub-maximal walking exercise (~58% $\dot{V}O_2$ peak). The implications for recreational and occupational groups may be far reaching, (for greater detail see Section 3.4.2).

A mechanism that may account for the observations here is the elevated work of breathing and impaired breathing mechanics imposed by chest wall loading and restriction. Exercise with load carriage increases the work and power of breathing through a curvi-linear increase in the force and velocity of contraction (Brown & McConnell, 2012a; Dominelli, Sheel, & Foster, 2012). In addition the restrictive component of the backpack limits operational lung volumes and reduces the efficiency of the respiratory muscles as their length tension relationship is altered (Dominelli et al., 2012). Consistent with previous findings (Muza, Latzka, Epstein, & Pandolf, 1989b; Richmond, Rayson, Wilkinson, Carter, & Blacker, 2008) a 4% reduction in FVC and 1% reduction in FEV_1 was observed at baseline when wearing the backpack (Table 3.2). Although small in magnitude, these changes have important consequences for the evolution of the exercise hyperpnoea response. During un-loaded breathing tidal volume increases with a simultaneous decrease in EELV which serves

to optimise diaphragm length and respiratory system compliance (Aliverti, 2008). However, during load carriage thoracic volume is reduced and the external limit placed upon the inspiratory reserve capacity lowers EILV and EELV, shifting the operational lung volume to a lower fraction of total lung capacity (Dominelli et al., 2012) reducing efficiency and increasing the work of breathing (Dominelli et al., 2012; Tomczak et al., 2011). Furthermore, subsequent reductions in EELV may also increase expiratory pressures at functional residual capacity, prompting an expiratory flow limitation (McClaran, Wetter, Pegelow, & Dempsey, 1999). Thus the greater prevalence of respiratory muscle fatigue with load carriage is most likely due to a combination of impaired breathing mechanics and elevated work of breathing during exercise. Support for this claim can be provided as a small subset of participants (n=4) stood stationary on the treadmill for 75 min whilst wearing the 25 kg load, there was no change in P_{Imax} (pre 167 ± 19 cmH_2O vs post 167 ± 21 cmH_2O) or P_{Emax} (pre 126 ± 3 cmH_2O vs post 120 ± 5 cmH_2O). Therefore it is suggested that during tidal breathing, the imposed constraints on EILV and EELV are not problematic; however, once the demand for alveolar ventilation is increased (during exercise) the imposed restriction of the backpack inhibits the required changes in lung volumes and mechanics, thus reducing mechanical efficiency and prompting respiratory muscle fatigue. It has also been suggested that a reduction in respiratory muscle pressure generation may also be a result of reflex inhibition and therefore reduced central motor drive (Marcora, 2009) rather than a reduction in capacity for the respiratory muscles to produce force. This was not apparent here as no change in handgrip strength pre vs post LC and LC_{TT} was observed in the same subset of participants (n=4). Therefore the reduction in P_{Imax} and P_{Emax} as indicative of global respiratory muscle fatigue rather than a reduction in whole body central motor drive. It is acknowledged within this study that RER values is abnormally high, this may be related to the timing of the

measurement, specifically as ventilatory parameters were measured immediately after exercise.

3.4.2 APPLIED RELEVANCE

Respiratory muscle fatigue identified here may have important consequences for whole body performance with respect to some occupational and recreational situations where thoracic load carriage is a prominent feature. Respiratory muscle fatigue has been shown to exacerbate limb muscle fatigue and impair high intensity whole body performance through a sympathetically-mediated reflex reduction in limb blood flow (known as the respiratory muscle metaboreflex) presumably in favour of the fatiguing respiratory muscles (Harms et al., 1997; Romer, Lovering, Haverkamp, Pegelow, & Dempsey, 2006). It is therefore suggested that chest wall loading and restriction caused by the positioning of the load on the thorax may reduce the critical threshold for respiratory muscle fatigue due to inhibited breathing mechanics. Furthermore, this process may also increase the perceptual response to exercise (Marcora, 2009) resulting in a reflex inhibition of central motor output from the cortical and/or sub-cortical centres to the locomotor muscles (Amann, 2011).

The diaphragm is also one of few muscles to insert directly on to the lumbar spine, and assists with maintaining trunk and spinal stability (Shirley, Hodges, Eriksson, & Gandevia, 2003). Thus diaphragm fatigue may promote trauma to the vertebral column, intervertebral discs, and/or spinal ligaments, all of which are common causes of low back pain and injury in soldiers carrying heavy loads upon the trunk (Knapik et al., 1996). The respiratory musculature is also tasked with preserving postural control such as preventing falling; such that it contracts autonomously in anticipation of actions that destabilise or load the trunk (Hodges, Butler, McKenzie, & Gandevia, 1997). This feed-forward control mechanism occurs irrespective of the phase of the breathing cycle; however, respiratory

function always takes precedence over its role in postural control (Hodges, Heijnen, & Gandevia, 2001). Consequently load carriage-induced respiratory muscle fatigue may compromise this and increase the risk of lower limb injury since this has been shown to shift the reliance from the respiratory musculature to the lower limbs and ankles for postural control (Janssens, Brumagne, Polspoel, Troosters, & McConnell, 2010) which is clearly important for activities where the terrain / topography is complex.

3.5 CONCLUSION

Load carriage (25 kg) during sub-maximal walking exercise caused significant respiratory muscle fatigue. In addition, the perceptual sensations and metabolic responses were increased and performance during a 2.4 km time trial was markedly reduced. These findings have important implications for some recreational and occupational groups. Given that the addition of the backpack load significantly impairs performance in part through a load carriage-mediated respiratory muscle fatigue, future research should investigate interventions to attenuate such effects and this was the purpose of the subsequent experimental chapters in this thesis.

CHAPTER 4

ACUTE INSPIRATORY MUSCLE LOADING DOES NOT IMPROVE RUNNING PERFORMANCE WITH THORACIC LOAD CARRIAGE

Presented at the European College of Sport Science annual congress

Malmö – Sweden 2015

4.1 INTRODUCTION

Whole body 'warm-ups' are integral in the preparation for exercise and such pre-exercise 'priming' has received considerable attention from the literature, clearly demonstrating an ergogenic effect (eg Bailey, Vanhatalo, Wilkerson, DiMenna, & Jones, 2009; Bishop, 2003a). Pre-exercise priming for endurance exercise can take a variety of forms (e.g. for psychological or other non-physiological outcomes). Specific physiological priming activities aim to improve performance by targeting large locomotor muscle masses to accelerate O_2 kinetics through a variety of central and peripheral mechanisms (for a comprehensive review see Bishop, 2003a, 2003b). It has been suggested however that priming activities pose little challenge to the respiratory muscles (Johnson et al., 2014; Tong & Fu, 2006; Volianitis, McConnell, Koutedakis, & Jones, 2001). The lack of emphasis upon respiratory muscle priming is surprising since the respiratory muscles constitute a fundamental link in respiration by powering the delivery of O_2 and CO_2 away from the gas exchange tissues of the lung (and hence preserve acid base balance). Furthermore, they contribute to blood flow distribution and the regulation of the perception of effort and central motor output during exercise (Dempsey et al., 2002; Marcora, 2009).

Previous research has demonstrated that acute inspiratory muscle loading increases the peripheral excitability of the diaphragm and intercostal muscles (Hawkes et al., 2007; Ross et al., 2007) resulting in transient increases in P_{Imax} (Ross et al., 2007).When combined with a whole-body warm-up, the transient improvement in inspiratory muscle strength provides an ergogenic effect to exercise performance. This has been demonstrated previously through increased time to the limit of tolerance in intermittent running (Lin et al., 2007; Lomax et al., 2011; Tong & Fu, 2006) and distance covered in a 6 min all out rowing time trial (Volianitis,

McConnell, Koutedakis, & Jones, 2001). Although, no ergogenic effect was observed when performing a 10 km cycling time-trial (Johnson et al., 2014).

Unlike unloaded exercise, carrying a thoracic load modifies breathing mechanics as discussed in detail in Section 1.4.4. Tidal volume becomes constrained via an inspiratory volume limitation (Dominelli et al., 2012) and these adjustments reduce respiratory muscle efficiency resulting in greater perceptions of respiratory, locomotor and whole-body effort. In addition, respiratory muscle fatigue is accelerated at intensities lower than that observed in unloaded exercise due to the greater load and force output of these muscles (~59% $\dot{V}O_2$ max, see Chapter 3). The ergogenic effect of inspiratory muscle priming has been attributed to the increased P_{Imax} which reduces the relative work intensity of the inspiratory muscles during exercise (Johnson et al., 2014; Lomax et al., 2011). This leads to reduced dyspnoea, lactate accumulation and inspiratory muscle fatigue (Lin et al., 2007; Lomax et al., 2011; Volianitis, McConnell, Koutedakis, & Jones, 2001). Given that the addition of load carriage to exercise exacerbates these variables and impairs performance suggests that when combined with appropriate whole-body pre-exercise priming, the physiological, perceptual and ergogenic effects of pre-exercise inspiratory muscle priming may be magnified.

Accordingly, the aim of this study was to investigate whether performance on a self-paced, 2.4 km time-trial with 25 kg thoracic load was improved after performing a period of acute inspiratory loading when combined with an active warm-up. It was hypothesised that time-trial performance would be improved and inspiratory muscle fatigue, perceptual and metabolic responses attenuated.

4.2 METHODS

Following ethics approval from the host University, nine healthy, non-smoking and physically active males (Table 4.1), familiar with load carriage through regular recreational load carriage activities, participated in this study. Following the participant preparation procedures outlined in Section 2.1, each participant visited the laboratory on seven separate occasions separated by a minimum of 72 hours rest.

Table 4.1 Descriptive characteristics of the participants (Mean ± SD; *n*=9).

Age (years)	26.4 ± 9.1
Body Mass (kg)	74.3 ± 10.8
Height (m)	1.7 ± 0.4
Body Fat (%)	21.3 ± 7.7
BIA Lean body mass (kg)	55.7 ± 4.9
BIA Fat mass (kg)	15.7 ± 7.2
$\dot{V}O_2$ peak ($L \cdot min^{-1}$)	3.52 ± 0.29
$\dot{V}O_2$ peak ($ml \cdot kg^{-1} \cdot min^{-1}$)	48.65 ± 6.71
V_{LTP} ($km \cdot h^{-1}$)	10.3 ± 1.6

Values are presented as mean ± SD. BIA bioelectrical impedance $\dot{V}O_2$ peak; maximal oxygen uptake in absolute units and relative to body mass; V_{LTP}, Velocity at lactate turnpoint.

4.2.1 PRELIMINARY TRIALS

Visits one and two were preliminary trials. In the first visit, an assessment of body composition was conducted using bioelectrical impedance analysis (Section 2.7.2). Participants then completed a maximal incremental running exercise test on a motorised treadmill (Desmo, Woodway, Germany) to determine both lactate threshold, lactate turnpoint (lactate profiling phase) and $\dot{V}O_2$ peak (maximal exercise phase) without the backpack. Lactate threshold was defined as the first increase in blood lactate concentrations from

baseline, and lactate turn point was defined as the second observable sustained deflection in blood lactate beyond lactate threshold (Eston & Reilly, 2009; Figure 4.1). To determine lactate threshold and velocity at lactate turn point, participants completed a 5 min warm-up at 8 km·h^{-1} and 1% gradient. Following the warm up the gradient was increased to 4% and the speed increased by 1 km·h^{-1} and the participant completed three minute stages. At the end of each three minute stage, participants dismounted the treadmill and blood lactate concentrations $[lac^-]_B$ were measured from arterialised-venous fingertip blood samples (Section 2.6) and analysed immediately using a lactate pro analyser (Accu-Check, Safe T-Pro, Birmingham, UK). Both lactate threshold and lactate turn point were identified through real-time visual inspection of each individual $[lac^-]_B$-velocity curve. Once velocity at lactate turn point (V_{LTP}) was identified, the intensity of the treadmill was increased by 1 km·h^{-1}·min^{-1} until the limit of volitional tolerance to determine $\dot{V}O_2$ peak (maximal exercise phase).

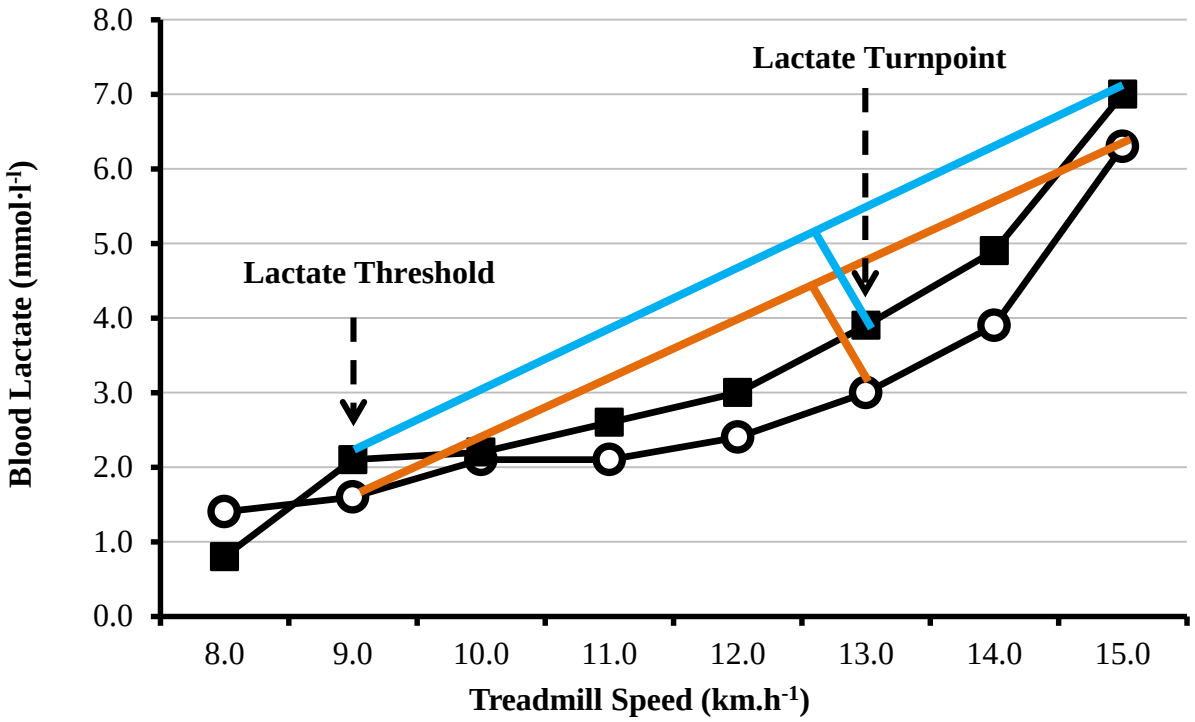

Figure 4.1 Two blood lactate response curves to incremental exercise, used for identification of both lactate threshold and velocity at lactate turnpoint.

During the second preliminary visit, participants were familiarised with all testing equipment and protocols. Participants then completed baseline pulmonary function (Section 2.4), maximal inspiratory and expiratory mouth pressure measurements, as detailed in Section 2.3. Following this, participants were fitted and familiarised with the 25 kg backpack (Web Tex, Bedford, UK) and completed a habituation 2.4 km time trial. The backpack was worn in accordance with the manufacturer's guidelines, incorporating adjustable shoulder straps and a waist strap which were adjusted individually and recorded to the nearest mm for subsequent trials.

4.2.2 EXPERIMENTAL TRIALS

Participants completed five experimental trials (Figure 4.2) randomised using a Latin square. Each trial comprised a 2.4 km time-trial (LC_{TT}) preceded by: 1) acute inspiratory muscle loading (IMWU); 2) placebo inspiratory muscle loading (PLA); 3) active warm-up (ACT), 4); ACT+IMWU, and 5) ACT+PLA. In contrast to Chapter 3, the time-trial was not pre-loaded with 60 min sustained steady state exercise since this would remove any potential ergogenic effects of prior inspiratory muscle loading as the physiological effects of this are ceased post 15 min (Ross et al., 2007).

The inspiratory warm up (IMWU) component required participant's to complete 2 × 30 dynamic inspiratory efforts using a pressure threshold loading device prescribed at 40% of P_{Imax} measured at the start of the experimental trial. After completion of the first 30 forced inspiratory efforts, P_{Imax} was re-assessed and the absolute intensity increased accordingly prior to the second bout. This protocol has been shown to increase diaphragm motor evoked potentials (Ross et al., 2007) and provide an ergogenic effect (Johnson et al., 2014; Lin et al., 2007; Tong & Fu, 2006; Volianitis et al., 2001). A placebo protocol (PLA) was also used, which saw the resistance spring removed and replaced with loosely packed aquarium gravel.

Participants were informed that the gravel was oxygen absorbent thereby reducing F_IO_2 to mimic hypoxia, causing prolonged post-warm-up compensatory O_2 extraction effect in the locomotor muscles during exercise resulting in an acute ergogenic effect. Participants were instructed to breathe normally during rest, through the device for a period of 5 min. All active (ACT) components comprised 10 min running without the backpack at V_{LTP} that was determined during preliminary assessments. The intensity and duration of all active components were determined via previous research that has shown that prior heavy exercise that elevates blood lactate to 3 mmol·l is effective in increasing the amplitude of the primary $\dot{V}O_2$ response and exercise performance during high intensity exercise (Burnley, Doust, & Jones, 2005). The duration was determined as exercise at ~60% $\dot{V}O_2$ peak for 10-20 minutes is sufficient to prevent depletion of high energy phosphates and fatigue whilst increasing muscle temperature and significantly improve short-term performance (Bishop, 2003a). During protocols that combined ACT with IMWU and PLA the active warm-up preceded the pressure threshold and placebo inspiratory muscle breathing tasks.

P_{Imax}, P_{Emax} and pulmonary function were measured at baseline, and immediately post LC_{TT} in each trial; P_{Imax} was also measured following all warm up procedures. Heart rate (Section 2.8.1), expired respiratory gasses (Section 2.5.2), $[lac^-]_B$ (Section 2.6) and perceptual responses (Section 2.8.2) were measured immediately prior to the warm-up, before LC_{TT}, at 0.4 km intervals during and in the last 0.1 km of $LC_{TT.}$ Following all pre-exercise routines participants donned the backpack and completed the 2.4 km time-trial.

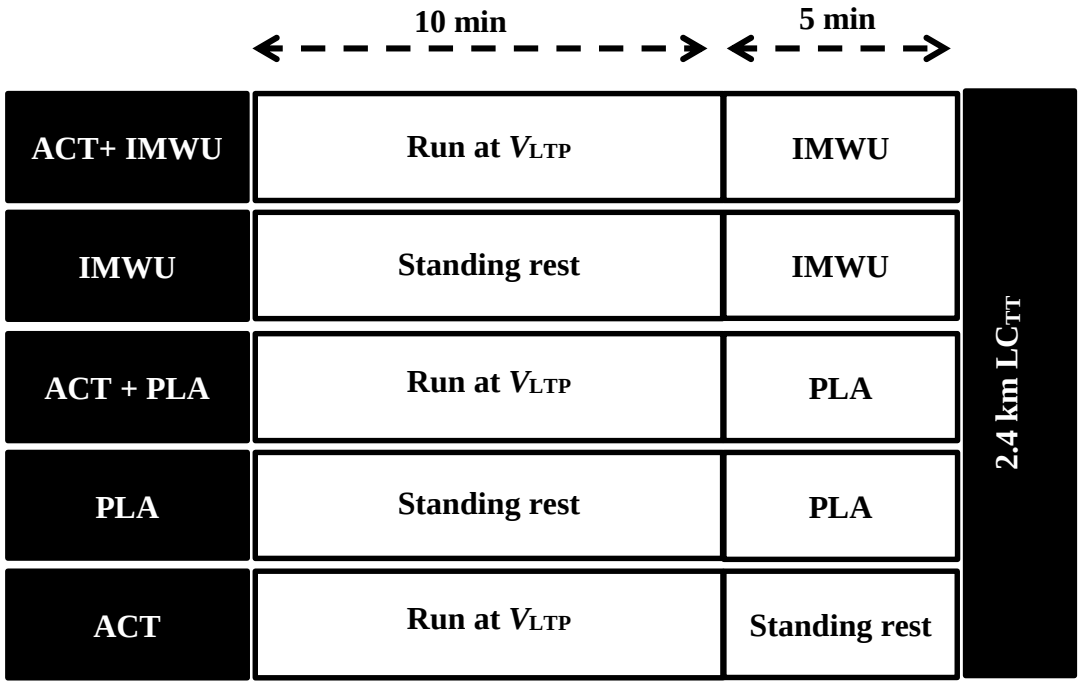

Figure 4.2 Schematic showing each of the five warm-up procedures and the components of each warm up; V_{LTP} velocity at lactate turn point, ACT active warm up, IMWU Inspiratory muscle warm-up and PLA placebo inspiratory muscle warm-up.

4.2.3 Statistical Analysis

Between trial differences were assessed using a 5 (warm-up conditions) x 1 (LC_{TT}) repeated measures ANOVA with a priori α at 0.05. Tukey's post hoc analysis was conducted using SPSS for Windows (SPSS, Chicago, IL, USA). Effect size was calculated using Cohen's *d* (where: $d=(\bar{x}^1-\bar{x}^2)$/pooled σ) and judgements of the magnitude of the effect were based on the 'minimal worthwhile effect' as described in previous literature (Hopkins, 2000). All results are presented as mean ± SD.

4.3 RESULTS

4.3.1 TIME-TRIAL PERFORMANCE

Performance on LC_{TT} was similar between each condition (see Figure 4.3, P>0.05). Mean running speed was also similar between trials (P>0.05) yet greatest during ACT+IMWU (10.33 ± 1.33 km·h^{-1}) followed by IMWU (10.30 ± 0.97 km·h^{-1}), ACT (10.22 ± 1.66 km·h^{-1}) ACT+PLA (10.01 ± 1.56 km·h^{-1}) and finally PLA (9.9+ ± 1.29 km·h^{-1}).

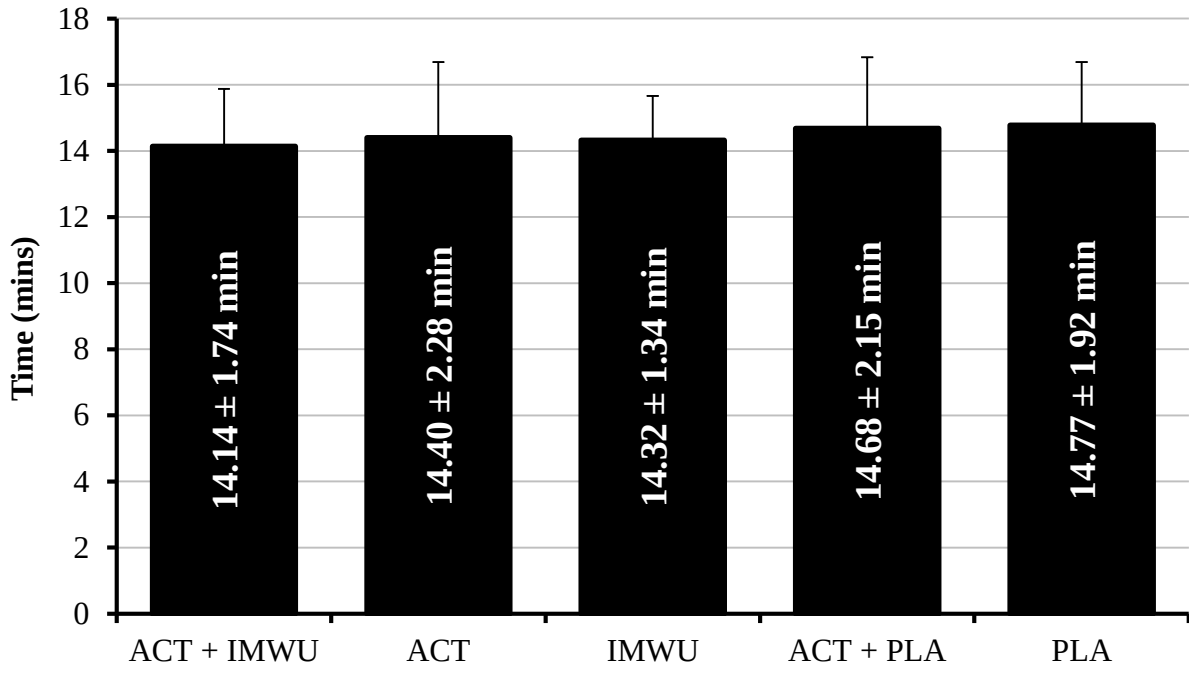

Figure 4.3 LC_{TT} performance time for each experimental condition. Trial abbreviations are defined above in Section 4.1.2.

4.3.2 RESPIRATORY MUSCLE PRESSURES

Baseline values of P_{Imax} and P_{Emax} were similar between all trials (P>0.05). Following IMWU there was a 7% increase in P_{Imax} (pre: 122 ± 25 cmH_2O vs post 136 ± 28 cmH_2O, P<0.05) and a 6% increase from baseline post ACT+IMWU (pre: 119 ± 26 cmH_2O vs post: 133 ± 27 cmH_2O, P<0.05) as shown in Figure 4.4. Following LC_{TT} P_{Imax} was reduced in all

experimental trials (pooled data: 9%, $P<0.05$) and this reduction was similar between trials (Figure 4.4). P_{Emax} was reduced from baseline in all trials to a similar magnitude (pooled data: 6%, $P>0.05$) (Table 4.2).

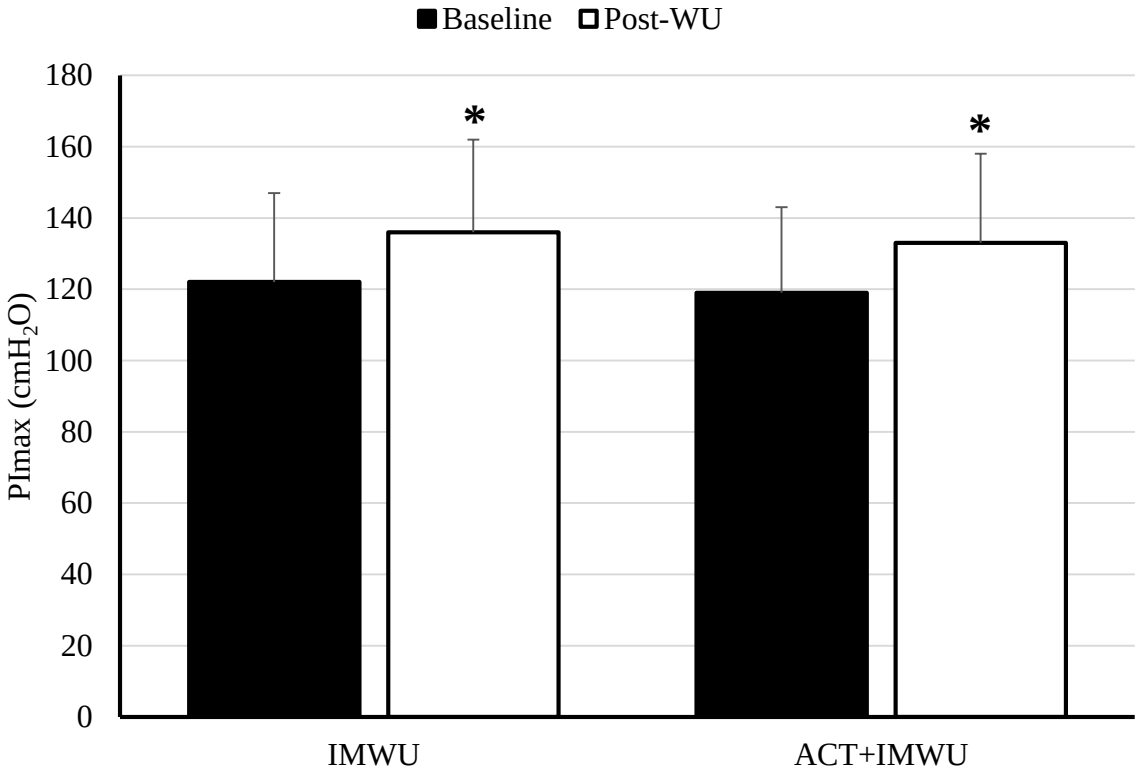

Figure 4.4 P_{Imax} at baseline and following IMWU and ACT+IMWU, * = different from baseline.

4.3.3 PULMONARY FUNCTION

Baseline and post LC_{TT} measures of pulmonary function that are averaged across all trials are shown in Table 4.2. Values were similar between trials at baseline and post LC_{TT} in all trials ($P<0.05$).

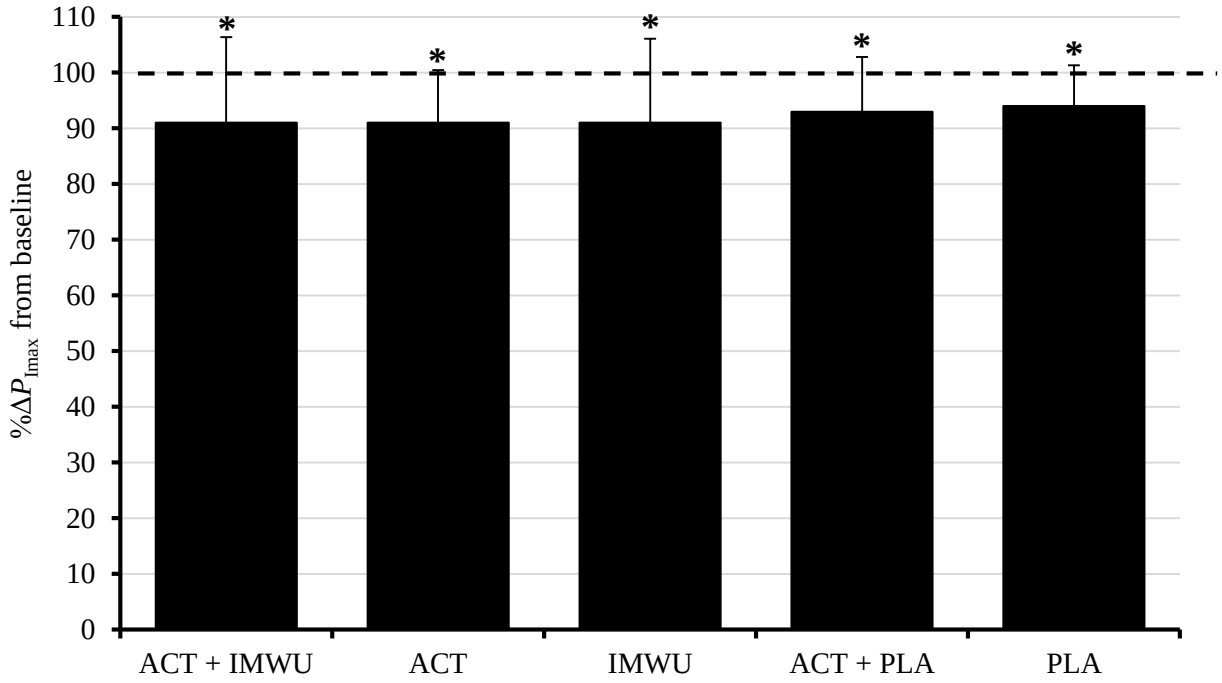

Figure 4.5 Relative change in inspiratory muscle pressure (P_{Imax}) from baseline following 2.4 km time-trial during each experimental trial. The dashed line represents baseline (100%), * pre- vs post time- trial reduction in $P_{Imax,}$ P<0.05.

4.3.4 PHYSIOLOGICAL AND PERCEPTUAL RESPONSES

Physiological data are presented in Table 4.2. All baseline values were similar between trials (P>0.05) and increased post warm-up after ACT+IMWU, ACT and ACT+PLA (P<0.05) and remained unchanged post warm-up in IMWU and PLA (P>0.05). Post LC_{TT} responses were greater than baseline and post warm-up in all trials (P<0.05); there were no between trial differences in any physiological or perceptual measure immediately following LC_{TT} (P>0.05).

Table 4.2 Mean physiological and perceptual responses at each time point within all trials.

	Baseline	**Post Warm up**	**Post LC_{TT}**
P_{Emax} (cmH_2O)	103 ± 26	-	100 ± 20
FEV_1 (L)	3.97 ± 0.31	-	3.92 ± 0.43
FVC (L)	3.97 ± 0.31	-	4.43 ± 0.46
FEV_1/ FVC (%)	82 ±	-	88 ± 6
PEF ($L \cdot min^{-1}$)	534 ± 69	-	497 ± 125
HR *(beats·min^{-1})*	82 ± 13	116 ± 37	193 ± 14
$[Lac^-]_B$ ($mmol \cdot l^{-1}$)	1.14 ± 0.38	1.91 ± 1.03	7.15 ± 1.23
$\dot{V}_E$ *(L·min^{-1})*	23.3 ± 11.1	77.2 ± 17.31	115.0 ± 18.8
f_B (breaths·min$^{-1)}$	24 ± 9	44 ± 10	61 ± 12
$\dot{V}O_2$ *(L·min^{-1})*	0.72 ± 0.31	2.44 ± 0.41	3.15 ± 0.48
$\dot{V}CO_2$ *(L·min^{-1})*	0.68 ± 0.33	2.55 ± 0.48	3.51 ± 0.54
RER	0.93 ± 0.11	1.04 ± 0.07	1.13 ± 0.08
RPE (AU)	6 ± 1	7 ± 1	16 ± 2
RPE_{legs} *(AU)*	0 ± 0	1 ± 0	7 ± 2
$RPE_{breathing}$*(AU)*	0 ± 0	1 ± 0	7 ± 2

Values are presented as mean ± SD. Maximum expiratory pressure (P_{Emax}), forced vital capacity (FVC), forced expiratory volume in one second (FEV_1), forced expiratory volume in one second / forced vital capacity ratio (FEV_1/ FVC), peak expiratory flow (PEF): Heart rate (HR), Blood Lactate $[Lac^-]_B$, minute ventilation ($\dot{V}_E$), oxygen consumption ($\dot{V}O_2$), carbon dioxide production (VCO_2), Respiratory exchange ratio (RER), Arbitrary units (AU).

4.4 DISCUSSION

The key findings of this study were fourfold. First: acute pressure threshold inspiratory muscle loading increased P_{Imax} after IMWU and ACT+IMWU ($P<0.05$). Second, despite the increase in P_{Imax} there was no improvement in performance relative to a matched placebo control ($P>0.05$). Third, there were no between trial differences for inspiratory muscle fatigue, physiological and perceptual variables ($P>0.05$). Finally, it is unlikely that acute inspiratory muscle loading would provide an ergogenic benefit to personnel operating in physically demanding occupations while carrying a 25 kg backpack and performing short duration high intensity exercise.

4.4.1 TIME TRIAL PERFORMANCE

Time-trial performance was faster (~9.7%) than in Chapters 2 and 3, which was due to the elimination of the 60 min pre-loading phase. Performance remained unchanged in all experimental trials in this study and although Figure 4.3 demonstrates that ACT+IMWU trial resulted in the fastest LC_{TT} (~4%, d=0.35), this change is within the 10.5% coefficient of variation for LC_{TT} (see Section 2.10.4.3.1) and is therefore most likely explained by the random variation of the protocol. To date, this is the only study to investigate the effects of acute inspiratory muscle loading upon time-trial performance while carrying a heavy thoracic load and clearly, the results can only be compared with other unloaded exercise studies which have employed a similar priming protocol.

Tong and Fu (2006) observed an increase in time to the limit of tolerance (19.5%) during an open ended intermittent running performance test when acute inspiratory loading was combined with an active warm-up, as outlined in greater detail below. Similar positive improvements were also reported from the same group when an identical pre-exercise priming routine preceded a badminton footwork performance test (8% improvement; Lin et al., 2007).

Lomax et al. (2011) investigated the use of concurrent acute and chronic loading strategies upon intermittent running performance and observed a 5% increase in intermittent run time to exhaustion when adopting the protocol of Tong and Fu (2006). In each of the studies described above, the authors incorporated a standardised active warm-up comprising 5 min self-paced treadmill running, 10 min of static stretching and finally, 5 min of self-paced running on a track. The IMWU was performed between static stretching and free running and was identical to the present methodology (Lin et al., 2007; Lomax et al., 2011; Tong & Fu, 2006). In studies employing cycling and rowing performance tests, which may be more comparable to the present study due to the modified breathing mechanics imposed by the exercise technique (Boussana et al., 2003), performance outcomes are mixed. Distances covered and mean power on a 6 min all out rowing simulation test was improved (3.2%) when combining a specific rowing warm up with IMWU. The active component mimicked pre-competition exercise and included 5 min of light jogging on a treadmill at a HR range of 110-130 beats·min^{-1}. This was followed by 10 min of stretching and finally 12 min of rowing with increasing intensity, achieved via alterations in stroke rate, concluding with 5 sprints at 30s, 45s and 60s at a stroke rate of 26-32 strokes·min^{-1}. Following a two minute rest to reflect the slight pause between the end of the warm-up and the beginning of the race, the time-trial began. Perceived dyspnoea and post exercise reductions in P_{Imax} were also reduced compared with a sub-maximal and specific rowing warm-up per se. In stark comparison Johnson et al. (2014) observed similar increases in P_{Imax} to others (Lin et al., 2007; Lomax et al., 2011; Tong & Fu, 2006; Volianitis, McConnell, Koutedakis, & Jones, 2001) following acute inspiratory loading. However, the addition of this to a 15 min cycling warm-up consisting of 3 x 5 min segments at 70, 80 and 90% of the velocity at the gas exchange threshold did not improve 10 km cycling time-trial performance. A significant difference in power output was observed during the first 4 - 5 km of the time-trial, where power was increased in the initial stages,

suggesting an alteration in pacing strategy due to the inspiratory muscle priming (Johnson et al., 2014). Consequently, the effects of combined IMWU and active warm-up upon performance remains open to interpretation and the potential mechanisms explaining this difference are explored below.

4.4.2 POTENTIAL PHYSIOLOGICAL EXPLANATIONS

Although the ergogenic effects of IMWU upon performance are variable, studies demonstrating an ergogenic effect of acute inspiratory muscle priming in combination with an active warm-up attribute the performance improvement to the increase in pre-exercise P_{Imax} (Lin et al., 2007; Lomax et al., 2011; Tong & Fu, 2006; Volianitis, McConnell, Koutedakis, & Jones, 2001). Greater inspiratory muscle strength occurs through an increased synchrony and motor unit coordination of these muscles and allows this muscle group to operate at a lower relative intensity during exercise (Hawkes et al., 2007), resulting in less inspiratory muscle fatigue, attenuated metabolite accumulation and dampened perceptions of effort (Bailey et al., 2010; Romer et al., 2006; Witt et al., 2007).

Only two previous studies have recorded measures of P_{Imax} pre and post exercise and hence assessed inspiratory muscle fatigue. Volianitis et al. (2001) demonstrated a 4% reduction in P_{Imax} when a rowing specific warm-up was combined with acute inspiratory loading compared to an 11% reduction when an active warm-up was performed alone. This is suggestive of attenuated inspiratory muscle fatigue (defined as a transient pre vs post-time trial attenuation in the reduction of P_{Imax}). This was likely due to a reduction in the cumulative inspiratory muscle work throughout exercise and therefore, the cardiovascular competition throughout exercise may also have been reduced leading to improved performance (Babcock et al., 2002; Dempsey et al., 2006). Inspiratory muscle fatigue was present after each time-trial in the present study to a similar magnitude as Volianitis and colleagues (2001, Figure,

4.4, $P>0.05$; pooled data: 11 ± 4%), although lower than in Chapters 3 and 4. However, a potential explanation for this difference between the present study and others is not forthcoming, but most likely due to the addition of the load and how this affects accessory respiratory muscle function (see below).

Studies have also demonstrated an attenuated metabolite response to exercise with IMWU. The present study demonstrated no change between trials in any physiological variable during the time-trial exercise in any experimental condition. This is difficult to resolve given the inspiratory muscles ability to affect lactate kinetics (Brown, Sharpe, & Johnson, 2010; Brown et al., 2012; Johnson et al., 2007) during unloaded exercise. In contrast to this, Lin et al. (2007) observed reduced blood lactate concentration following acute inspiratory loading which they associated to increased blood flow to the inspiratory muscles, although empirical evidence of this is lacking. Johnson et al. (2014) also demonstrated numerous changes in physiological parameters during the initial stages of a 10 km cycling time-trial, however, changes normalised between trials during the closing stages of the test. Acute loading of the inspiratory muscles may facilitate greater metabolite uptake and/or reduced metabolite production by accelerating oxidative metabolic pathways in this muscle group (Brown et al., 2010, 2012); however, their specific ability to affect systemic physiology after acute loading is yet to be determined.

Finally, studies have demonstrated attenuated perception of breathing discomfort during exercise (Lin et al., 2007; Tong & Fu, 2006; Volianitis, McConnell, Koutedakis, & Jones, 2001), and it has been suggested that the lower relative intensity of inspiratory contractions alter the firing rate of group III and IV muscle afferents (Witt et al., 2007), reducing their sensory transmission to the sensorimotor cortex. The modified perceptual template and conscious effort experienced during exercise (Johnson et al., 2014; Tucker,

2009) may raise the threshold of an acceptable level of effort during exercise and improve performance. In addition, reduced conscious effort during exercise has also been associated with sustained levels of arousal during exercise which can benefit performance (Hampson, Gibson, Lambert, & Noakes, 2001) although this only appears to be beneficial in the short term and has no sustained performance benefit (Johnson et al., 2014). Despite this potential mechanism and others, this appears to provide no benefit to exercise while carrying a thoracic load. Accordingly, our findings may therefore be explained by the nuances of design of previous research and/or the effects of load carriage per se upon respiratory mechanics, both of which are explored below.

4.4.3 PRIOR RESEARCH DESIGN LIMITATIONS

Despite the ergogenic effects of pre-exercise acute inspiratory muscle priming and the physiological and perceptual changes reported previously in some studies (Lin et al., 2007; Lomax et al., 2011; Tong & Fu, 2006; Volianitis, McConnell, Koutedakis, & Jones, 2001), the present study demonstrated no change in time-trial performance in any experimental condition and no change in inspiratory muscle fatigue, physiological or perceptual variables. The findings here are similar to those of Johnson et al. (2014) who also demonstrated no change in 10 km time-trial performance when acute inspiratory loading was added to an active warm-up. The mechanisms explaining these differences are not fully understood and do not appear to be explained by the performance test used. Previous studies that observed improvements in performance employed either open ended fixed intensity exercise (Lin et al., 2007; Lomax et al., 2011; Tong & Fu, 2006) or fixed duration all out (6 min) exercise. The present study and that of Johnson et al. (2014) employed fixed-distance time-trial exercise and demonstrated no change in performance. Although very different in their design, the ventilatory demand of fixed duration and time-trial exercises are likely to be similar (Dempsey et al., 2014) and increased ventilatory demand does not affect performance (Romer

et al., 2006). However, differences in performance outcomes may be explained by the design of the warm-up protocol prior to the performance task (Johnson et al., 2014). In the present study and that of Johnson et al., (2014), the active component of the warm-up was prescribed for each individual relative to the lactate turnpoint or gas exchange threshold, which produced a similar ventilatory challenge ~80 $L \cdot min^{-1}$ (see Table 4.2). Others, however, instructed participants to complete a self-paced jog either on a treadmill or in an open space such as a sports hall or running track (Lin et al., 2007; Lomax et al., 2011; Tong & Fu, 2006). It has been demonstrated that such warm-ups are often sub-optimal and can be detrimental to exercise performance as the intensity can be either below or above the optimum intensity which demarcates steady state and non-steady state exercise (Ingham, Fudge, Pringle, & Jones, 2013). The intensity of the active warm-up was controlled in the present study at V_{LTP} in agreement with previous recommendations for short duration (<20 min) intense exercise (Bishop, 2003a). Johnson et al., (2014) also fixed the relative intensity of each incremental element of the active warm-up and concluded that an appropriately designed active warm-up at a fixed relative intensity adequately primes the inspiratory muscles for exercise. The ventilatory demand of previous studies was not reported which makes it difficult to compare to the present work. It has been suggested that active warm ups that are self-paced are sub-optimal, and pose little challenge to the respiratory musculature (Johnson et al., 2014; Lomax et al., 2011) and the data presented in this chapter supports this notion.

4.4.4 Load Carriage effects upon respiratory mechanics

The evidence supporting the ergogenic effect of prior-inspiratory muscle priming is equivocal. However, given the impaired breathing mechanics associated with load carriage and the prevalence of inspiratory muscle fatigue during the 2.4 km time-trial that was demonstrated in Chapters 3 (and subsequently later in this thesis in chapters 5 and 6), it is paradoxical that performance was not improved in any trial. Accordingly, there appears to be

some other mechanism regulating performance during load carriage exercise, which is independent of the transient increase in P_{Imax} and cascade of potential physiological changes. As explained in detail in Chapter 1, the inspiratory muscles perform a dual role during exercise powering ventilation and supporting spinal stability with the latter magnified considerably during load carriage tasks. Acute inspiratory muscle loading targets the muscles of the chest wall and the diaphragm. It appears that this stimulus must not be great enough to attenuate the inspiratory muscles role of contributing to spinal stability or trunk control. It may also not extend to those accessory/synergistic inspiratory muscles responsible for holding/controlling the mass of the load upon the thorax. Interestingly, there was no change in performance when comparing the active warm-up per-se relative to the IMWU. This suggests that during the load carriage, the mass of the backpack and the muscular work required by the primary and/or accessory respiratory muscles in stabilising the spine and mechanical load, far outweighs and even regulates performance above that of acute locomotor muscle pre-exercise priming.

However, despite acute inspiratory muscle loading not affecting cycling time-trial performance (Johnson et al., 2014), chronic inspiratory muscle loading (i.e. IMT) does appear to provide a consistent ergogenic effect (Romer et al., 2002a) due to the specific functional, structural and morphological adaptations of this muscle group (for a comprehensive review see: (HajGhanbari et al., 2013; Illi et al., 2012). Therefore it is attractive to speculate that chronic loading of the inspiratory muscles may well provide a strong enough stimulus to the inspiratory muscle; thus providing an ergogenic aid during exercise with load carriage. This may occur by potentially affecting inspiratory muscle fatigue, systemic physiology and/or by reducing/off-loading the contribution of the respiratory muscles to their spinal stability tasks.

4.5 CONCLUSION

Acute inspiratory muscle loading increased P_{Imax} due to changes in central and peripheral neural efficiency. This increase provided no ergogenic effect to load carriage time-trial performance. Accordingly, the transferable benefit of acute inspiratory muscle loading to performance in occupational settings is questionable due to the intensity of exercise is high and low exercise duration tasks. However, the ergogenic effect of chronic loading of the inspiratory muscles, providing structural adaptations to this muscle group, is considerable and provides an interesting avenue for future study. Accordingly, this was the aim of the subsequent chapter.

CHAPTER 5

CHRONIC INSPIRATORY MUSCLE LOADING IMPROVES RUNNING PERFORMANCE WHEN CARRYING A 25 KG THORACIC LOAD

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5.1 INTRODUCTION

The consequence of bearing external loads in a backpack which alters breathing mechanics and induces respiratory muscle fatigue at submaximal intensities, as observed in Chapters 3 and 4. This reduces whole-body performance by attenuating locomotor muscle blood flow and heightening perception of effort when compared with unloaded activities (Dempsey, Romer, Rodman, Miller, & Smith, 2006; Harms et al., 1997).

Inspiratory muscle fatigue has been documented after constant power, high intensity, short duration exercise (10 to 15 min) to the limit of volitional tolerance (~85% $\dot{V}O_2$ peak) (Dempsey et al., 2006), high-intensity time trial exercise (Johnson, Sharpe, & Brown, 2007; Romer, McConnell, & Jones, 2002b) and prolonged (120 to 180 min) submaximal exercise such as marathon running (Ross, Middleton, Shave, George, & McConnell, 2008). In an occupational context however, there is a dearth of literature exploring the consequences of thoracic loads upon respiratory muscle function. Previous research has observed reductions in maximum respiratory pressures following sub-maximal and intense treadmill exercise while wearing self-contained breathing apparatus (SCBA) and fire-fighter apparel (Butcher, Jones, Eves, & Petersen, 2006). However, SCBA incorporates a gas regulator, (similar to those worn by firefighters), delivering high-pressure air (~5 cmH_2O) from compressed-air cylinders and it is difficult to distinguish the effects of the load mass secured to the thorax (from the gas cylinders) and the positive pressure provided by the face mask since the latter accounts for the majority of the increased work of breathing experienced during exercise (Eves, Jones, & Petersen, 2005).

Exercise in physically demanding occupations, comprises low intensity steady state exercise (e.g., during military patrol) and high intensity exercise (e.g., during military engagement). In a military context, carrying a 25 kg thoracic load it has previously been demonstrated that an 11% reduction in P_{Imax} following moderate duration (60 min) low

intensity treadmill walking (~58% $\dot{V}O_2$ peak) which further decreased 5% following a high-intensity 2.4 km self-paced time-trial (See Section 2.10.3.3 and Chapter 3).

In contrast to acute inspiratory muscle loading (adopted in the previous chapter), has no impact upon load carriage performance and a mixed effect upon inspiratory muscle fatigue and performance during unloaded exercise. Chronic inspiratory muscle loading or as commonly known as inspiratory muscle training (IMT, see Section 1.3.2) has been employed to affect inspiratory muscle fatigue and performance (for a review see HajGhanbari et al., 2013; Illi, Held, Frank, & Spengler, 2012). IMT increases the strength of the chest wall inspiratory muscles and the diaphragm (Brown, Johnson, & Sharpe, 2014) with exercise-induced inspiratory muscle fatigue either attenuated (Romer & McConnell, 2004) or unchanged (Verges, Lenherr, Haner, Schulz, & Spengler, 2007). IMT also improves whole-body time-trial performance in cycling (Johnson et al., 2007), running (Tong et al., 2008) and rowing (Griffiths & McConnell, 2007). The effect of IMT upon inspiratory muscle fatigue following exercise with load carriage and the effects of this upon load carriage performance remains unknown. Accordingly the aim here was to investigate the effects of 6 wk IMT upon physiological and perceptual responses during 60 min submaximal walking exercise and upon 2.4 km self-paced running time-trial while carrying a 25 kg load. It was hypothesised that IMT would increase inspiratory muscle strength; reduce inspiratory muscle fatigue and in turn, improve time trial performance.

5.2 METHODS

5.2.1 PARTICIPANTS

Following ethics approval from the host University and written informed consent, 19 healthy, non-smoking and physically active males fully habituated with load carriage through regular recreational load carriage activities completed a double-blind placebo controlled intervention. Participants were randomly assigned to either an IMT group (*n=10*) and a placebo group (PLA; *n=9*) following the first experimental trial (Table 5.1). Initially, PLA comprised 10 participants; however, one withdrew from the study due to personal reasons. Prior to the study participants were fully briefed on all procedures as outlined in Section 2.1 and there were no between group differences in any variable ($P>0.05$).

5.2.2 EXPERIMENTAL DESIGN

5.2.2.1 PRELIMINARY TRIALS

Participants completed two preliminary trials separated by a minimum of seven days. First, body composition was assessed as described in Section 2.7.1, followed immediately by a maximal incremental exercise test to determine peak O_2 uptake ($\dot{V}O_2$ peak) as described in Section 2.10.1. During the second preliminary visit participants were familiarised with all testing equipment and protocols and completed baseline pulmonary function (Section 2.4.1), maximal inspiratory (P_{Imax}) and expiratory pressure (P_{Emax}) measurements as detailed in Section 2.3. During the second preliminary trial, participants were familiarised and fitted with the 25 kg backpack (Web Tex, Bedford, UK) and completed 20 min exercise at 0% gradient and 6.5km·h-1. Following 15 min of seated recovery participants then completed a self-paced 2.4 km time-trial

Table 5.1 Mean ±SD for the descriptive characteristics of the participants (n=19).

Descriptive Characteristic	IMT (*n*=10)	PLA (n=9)
Age (years)	25.8 ± 8.7	24.2 ± 6.3
Body Mass (kg)	77.4 ± 9.3	78.1 ± 7.7
Height (m)	1.74 ± 0.04	1.81 ± 0.08
P_{Imax}(cmH_2O)	141 ± 28	143 ± 34
P_{Emax} (cmH_2O)	164 ± 33	160 ± 37
FEV_1 (L)	3.6 ± 0.5	3.9 ± 0.5
FVC (L)	4.7 ± 0.5	5.0 ± 0.9
FEV_1/ FVC (%)	79 ± 8	84 ± 8
PEF ($L \cdot min^{-1}$)	474 ± 106	539 ± 90
Body Fat (%)	21.9 ± 6.1	24.4 ± 5.1
Lean body mass (kg)	59.6 ± 5.6	58.0 ± 4.7
Fat mass (kg)	17.8 ± 6.0	20.1 ± 5.3
Bone mineral density (g/cm^2)	1.3 ± 0.2	1.3 ± 0.2
Bone mineral content (kg)	3.2 ± 0.5	3.2 ± 0.6
$\dot{V}O_2$ peak ($L \cdot min^{-1}$)	4.3 ±0.5	4.1 ± 0.4
$\dot{V}O_2$ peak ($ml \cdot kg^{-1} \cdot min^{-1}$)	52.6 ± 5.7	49.2 ± 4.5
Training Adherence (%)	93.8 ± 7.9	90.5 ± 11.2

Values are presented as mean ± SD. P_{Imax}, maximal inspiratory pressure, P_{Emax}, maximal expiratory pressure, FVC, forced vital capacity, FEV1, forced expired volume in one second, PEF, peak expiratory flow; $\dot{V}O_2$ peak; maximal oxygen uptake in absolute units and relative to body mass.

5.2.2.2 EXPERIMENTAL TRIAL

Participants completed the experimental protocol as described below on two occasions separated by a six wks intervention (IMT or PLA). Participants walked for 60 min, 0% gradient and $6.5 km \cdot h^{-1}$ carrying a 25 kg backpack (LC). Following 15 min of seated recovery participants then completed a 2.4 km self-paced time trial (LC_{TT}), the speed of the treadmill here was manually adjusted by the individual to complete the distance in the quickest time possible. Mouth pressure measures and pulmonary function were conducted at rest and

immediately after both LC and LC_{TT} as described in Sections 2.3 and 2.4, respectively. Physiological parameters were measured during the first minute of exercise and at 15min intervals throughout LC and also prior to, after 1.2 and 2.4km of LC_{TT}. Due to the length of time between each experimental trial, participants repeated the shortened familiarisation session one week prior to the experimental trial to ensure familiarity.

5.2.2.3 EQUIPMENT AND MEASUREMENTS

Baseline measures were conducted prior to exercise as outlined previously and included resting: volitional mouth pressures (Section 2.3), pulmonary function (Section 2.4), blood samples (Section 2.6.1), pulmonary ventilation (Section 2.5.1), heart rate (Section 2.8.1), perceptual responses (Section 2.8.2) which were repeated immediately Post LC and Post LC_{TT}.

5.2.3 TRAINING INTERVENTION

On completion of the first LC trial, using a double-blind procedure, participants were randomly assigned to either an IMT or Sham PLA group which were matched for LC_{TT} time and completed either specific IMT or placebo control. IMT consisted of thirty dynamic inspiratory efforts against a pressure-threshold load of 50% P_{Imax}, twice daily for 6 wk. throughout the training intervention; subjects were instructed to periodically increase the load to a threshold pressure which would permit them to complete only 30 breaths. Each inspiratory manoeuvre was initiated from residual volume and subjects strove to maximise V_T. To avoid hypocapnia, subjects were instructed to expire slowly and fully, thus reducing f_B, which is known to be effective in eliciting an adaptive response (Brown et al., 2014, 2010, 2012; Johnson et al., 2007); Section 1.3.2). Throughout the intervention period, subjects completed a training diary to record IMT adherence and habitual training (Appendix 3).

The placebo device was identical to the IMT device, however the resistance spring was removed and replaced with loosely packed aquarium gravel with participants informed that the gravel was oxygen absorbent thereby reducing F_IO_2 to mimic altitude training. Participants were instructed to breathe normally, while at rest, through the device for a period of 15 min, once daily, five days·wk^{-1}. This PLA protocol has been well documented to maximise participant expectation and motivation, yet cause no change in inspiratory muscle strength or changes in time-trial performance (Johnson et al., 2007; Sonetti, Wetter, Pegelow, & Dempsey, 2001). As with the IMT device P_{Imax} was assessed at two week intervals during which time the PLA gravel was replaced.

5.2.4 Statistical Analysis

Between subject differences and interaction effects for dependent variables were assessed using a 2 (group: IMT vs. PLA) x 3 (time point: rest, post-LC, post-LC_{TT}) x 2 (Trial pre vs. post intervention) mixed model ANOVA with Tukey's post hoc analysis using SPSS for Windows (Chicago, IL, USA). A priori α was set at 0.05. All results are presented as mean ± SD. Effect size was calculated using Cohen's *d* ($d=(x^1–x^2)$/pooled σ), judgements were made on the magnitude of the observed effect based on the 'minimal worthwhile effect' as described in previous literature (Hopkins, 2000).

5.3 RESULTS

5.3.1 TIME-TRIAL PERFORMANCE

Pre and post-intervention LC_{TT} time for both groups are shown in Figure 5.1.Time-trial performance improved post-IMT (absolute reduction = 1.3 ± 0.7 min, 8 ± 4%, group x trial interaction effect: $p<0.05$; effect size: $d=0.93$), and was unchanged in PLA. The mean speed during LC_{TT} increased after IMT (2.6 ± 0.3 $m \cdot s^{-1}$ vs 2.8 ± 0.4 $m \cdot s^{-1}$, $p<0.05$; effect size: $d=0.86$) and was unchanged after PLA (pre 2.5 ± 0.3 $m \cdot s^{-1}$ vs post 2.5 ± 0.4 $m \cdot s^{-1}$; $p>0.05$).

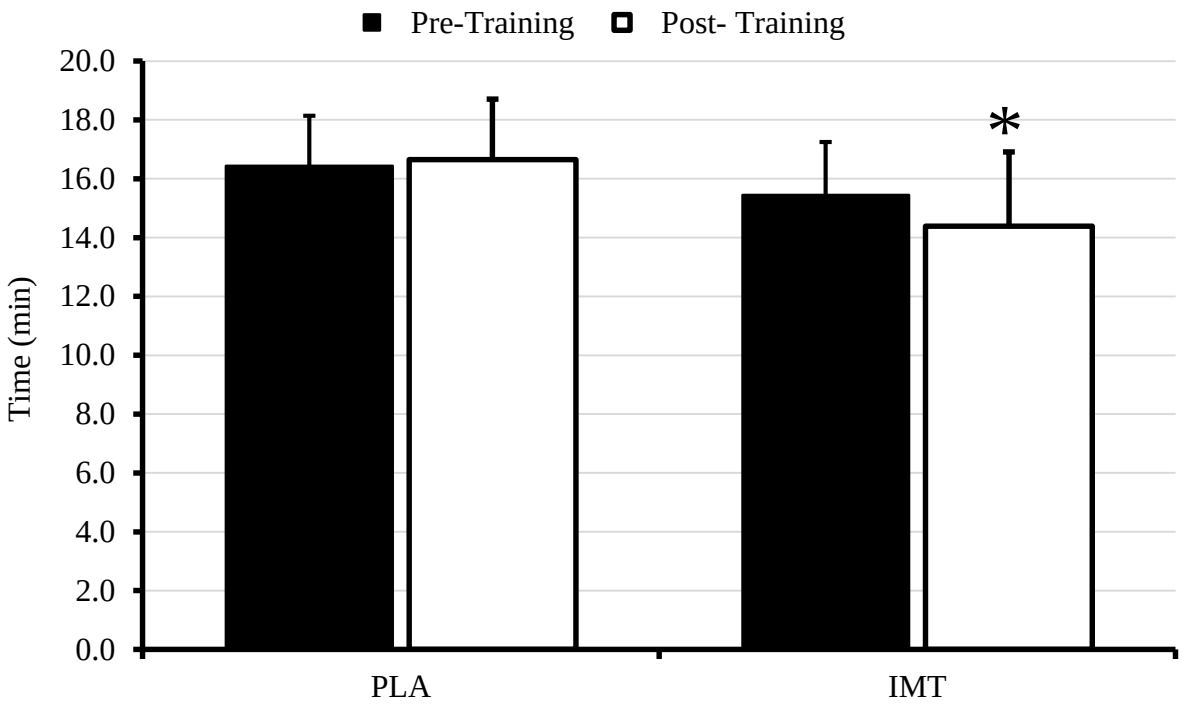

Figure 5.1 LC_{TT} performance pre and post intervention in both IMT and PLA. *, different between trials ($P< 0.05$).

5.3.2 RESPIRATORY MUSCLE PRESSURES

Training compliance was high in both groups (IMT 94 ± 8% and PLA 91 ± 11%) and was similar to previous IMT studies using the same protocol (Brown et al., 2012; Johnson et al., 2007). Individual changes in maximal P_{Imax} post IMT and post PLA are shown in Figure 5.2,

increase in P_{Imax} was also correlated with increase in LC_{TT} performance in IMT only (r= 0.738, P<0.05). Baseline and changes between pre and post-intervention for P_{Imax} and P_{Emax} are shown in Figure 5.1. Prior to the intervention, relative to resting values, P_{Imax} and P_{Emax} were reduced post-LC and post LC_{TT} in both groups (pooled data, P_{Imax}: 13 ± 7% and 16 ± 8%. P_{Emax}: 14 ± 7% and 17 ± 16%; P<0.05) which was similar post intervention in PLA. Relative to pre-IMT, P_{Imax} was greater at each time-point: baseline (+31%, P <0.05), post-LC (+19%, P<0.05) and post-LC_{TT} (+18%, P<0.05). Relative to pre-IMT, P_{Emax} was also greater at each time-point: baseline (+17%, P<0.05), post-LC (+15%, P<0.05) and post-LC_{TT} (+7%, P<0.05). However, although P_{Imax} was greater at each time point after IMT, the reductions relative to baseline were similar to pre-intervention after the 60 min steady state phase (pre IMT %Δ P_{Imax}: 11.3 ± 6.8% vs post IMT 12.8 ± 10.8%; P>0.05) and the time trial (pre IMT %Δ P_{Imax}: 2.3 ± 10.2 % vs post IMT 5.3 ± 12.7%; P>0.05). Reductions in P_{Emax} were greater compared with pre-intervention (pre IMT %Δ P_{Emax}: 5.5 ± 7.8% vs post IMT 11.7 ± 7.8%; P<0.05) and similar post LC_{TT} (pre IMT %Δ P_{Emax}: 18.6 ± 6.2 % vs post IMT 21.6 ± 12.2%; P>0.05).

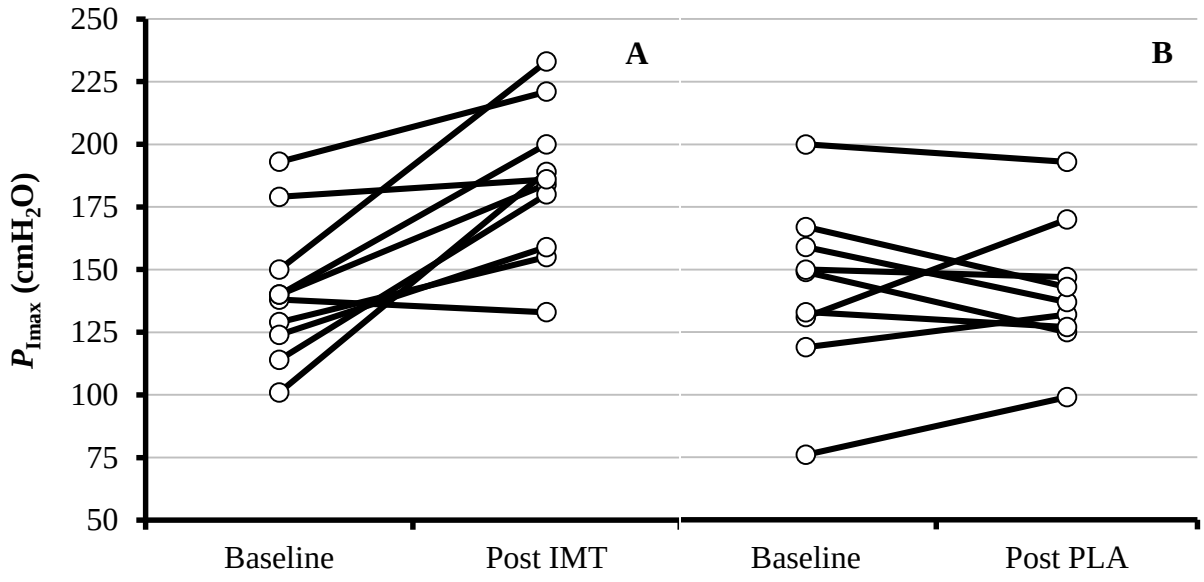

Figure 5.2 Individual changes in maximal inspiratory pressure (P_{Imax}) from baseline, post IMT (panel A) and post PLA (panel B).

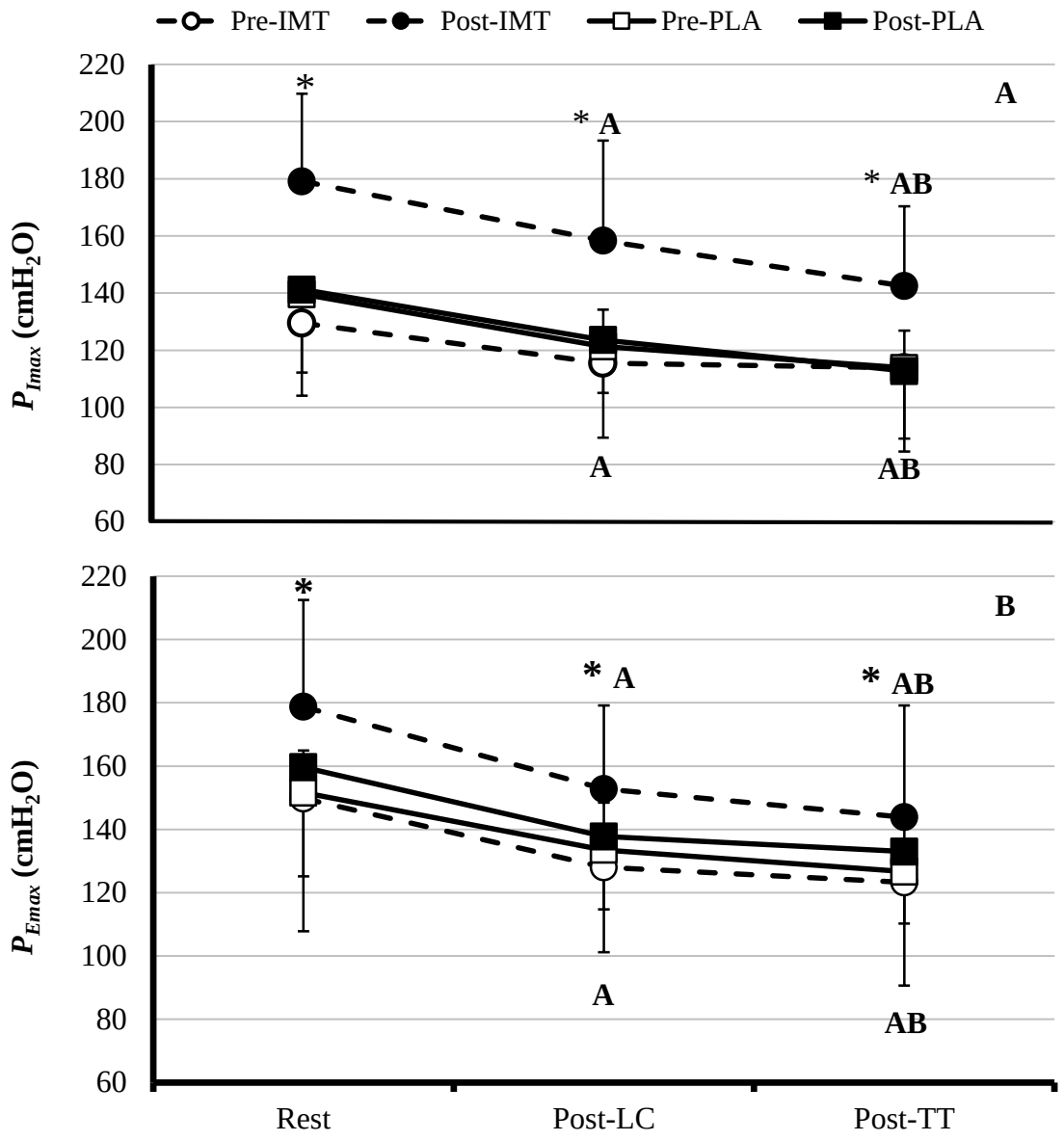

Figure 5.3 Between and within group changes in $P_{I\max}$ (panel A) and $P_{E\max}$ (panel B) prior to and following inspiratory muscle training. The circle markers represent the IMT training group and the squares represent the placebo group, values are presented as mean ± sd. [A], different to baseline: [B], different to Post-LC; *, Different between groups.

5.3.3 PULMONARY FUNCTION

Baseline and changes between trials were similar between groups prior to and post intervention (p<0.05), and remained unchanged following the intervention.

5.3.4 PULMONARY GAS EXCHANGE

Gas exchange responses to the experimental trial for the IMT and PLA groups are shown in Tables 5.2 and 5.3, respectively. Prior to the intervention with the exception of RER and one measure of $\dot{V}O_2$ (post-LC_{TT}, pre-intervention, IMT group) minute ventilation ($\dot{V}_E$), $\dot{V}O_2$ and carbon dioxide production ($\dot{V}CO_2$) was greater than rest post-LC and post-LC_{TT} (P<0.05), with no between group differences (P>0.05). Following the intervention, similar values were observed at baseline and over time for all variables in both groups.

5.3.5 PHYSIOLOGICAL RESPONSES

Baseline values of HR were similar between groups at rest (Table 5.2 and 5.3). HR increased from rest, post-LC (pooled data: post-LC, 31 ± 10%; P<0.01) and post-LC_{TT} (pooled data: 45 ± 8%; P<0.01) and also increased between post-LC to post-LC_{TT} (23 ± 9%; P<0.05); prior to the intervention HR was not different between groups (P>0.05). Following the intervention HR was significantly lower (7%) post-LC in IMT (P<0.05), relative to the same time point and lower (6%) than PLA (P<0.05). Both $[lac^-]_B$ and $[Glucose]_B$ were similar between groups at rest and over time before and after the intervention (P>0.05).

5.3.6 PERCEPTUAL RESPONSES

Changes in perceptual responses for IMT and PLA are shown in Tables 5.2 and 5.3, respectively. Prior to the intervention all perceptual responses were similar at baseline between groups (P>0.05), and increased from rest, at both post-LC (P<0.01) and post-LC_{TT} (P<0.01) with no differences between groups (P>0.05). Post intervention responses were similar in PLA but whole-body RPE (8%) and RPE_{legs} (10%) were lower post-LC in IMT (P<0.05).

Table 5.2 Responses to 60 min load carriage (LC) and 2.4 km load carriage time trial (LC_{TT}) prior to and following IMT (n=10).

	Pre-IMT			Post-IMT		
Variable	**Pre-LC**	**Post-LC**	**Post-TT**	**Pre-LC**	**Post-LC**	**Post-TT**
HR *(beats·min^{-1})*	102 ± 16	145 ± 19^{A}	187 ± 15^{AB}	108 ± 15	135 ± 29^{A*C}	193 ± 7^{AB}
$[Lac^-]_B$ (mmol·l^{-1})	1.5 ± 1.1	1.3 ± 0.6	8.5 ± 4.2^{AB}	1.5 ± 0.4	1.3 ± 0.6	9.6 ± 3.2^{AB}
$\dot{V}_E$ *(L·min^{-1})*	40.8 ± 8.2	46.2 ± 9.2^{A}	62.4 ± 22.6^{AB}	37.2 ± 11.3	46.6 ± 6.7^{A}	79.1 ± 25.5^{AB}
$\dot{V}O_2$ *(L·min^{-1})*	1.49 ± 0.35	1.66 ± 0.30	1.69 ± 0.54	1.27 ± 0.40	1.74 ± 0.31 ^{A}	1.68 ± 0.76
$\dot{V}CO_2$ *(L·min^{-1})*	1.40 ± 0.28	1.72 ± 0.27 ^{A}	1.71 ± 0.55	1.14 ± 0.43	1.60 ± 0.29 ^{AB}	2.06 ± 0.51 ^{AB}
RER	0.95 ± 0.14	1.05 ± 0.15	1.64 ± 0.75	0.89 ± 0.21	0.93 ± 0.12	1.33 ± 0.33 ^{A}
RPE (AU)	7 ± 1	12 ± 2^{A}	16 ± 3^{AB}	6 ± 0	11 ± 3^{A*}	17 ± 3^{AB}
RPE_{legs} *(AU)*	0 ± 0	4 ± 1^{A}	7 ± 1 ^{AB}	0 ± 0	3 ± 2*	7 ± 2 ^{AB}
$RPE_{breathing}$*(AU)*	0 ± 0	3 ± 2^{A}	7 ± 2 ^{AB}	0 ± 0	3 ± 2^{A}	8 ± 2 ^{AB}

Table 5.3 Responses to 60 min load carriage (LC) and 2.4 km load carriage time trial (LC_{TT}) prior to and following PLA (PLA, n=9).

	Pre-PLA			Post-PLA		
Variable	**Pre-LC**	**Post-LC**	**Post-TT**	**Pre-LC**	**Post-LC**	**Post-TT**
HR *(beats·min^{-1})*	96 ± 10	140 ± 20^{A}	190 ± 11^{AB}	103 ± 13	144 ± 20^{A}	184 ± 23^{AB}
$[Lac^-]_B$ (mmol·l^{-1})	1.7 ± 0.5	1.8 ± 1.1	8.8 ± 2.8 ^{A}	1.7 ± 0.5	1.6 ± 0.5	8.5 ± 3.1 ^{A}
$\dot{V}_E$ *(L·min^{-1})*	39.5 ± 7.5	50.6 ± 6.5^{A}	61.0 ± 23.9^{AB}	34.2 ± 12.2	48.6 ± 5.3^{A}	78.1 ± 30.1^{AB}
$\dot{V}O_2$ *(L·min^{-1})*	1.65 ± 0.30	1.82 ± 0.15	1.70 ± 0.79	1.31 ± 0.57	1.76 ± 0.23^{A}	1.52 ± 0.28
$\dot{V}CO_2$ *(L·min^{-1})*	1.46 ± 0.26	1.76 ± 0.26	1.74 ± 0.77	1.14 ± 0.48	1.64 ± 0.30^{A}	2.03 ± 0.70^{AB}
RER	0.89 ± 0.11	0.97 ± 0.15	1.24 ± 0.38 ^{A}	0.93 ± 0.21	0.95 ± 0.10	1.32 ± 0.35^{AB}
RPE (AU)	6 ± 1	11 ± 3^{A}	17 ± 2 ^{AB}	7 ± 1	11 ± 3^{A}	17 ± 3^{AB}
RPE_{legs} *(AU)*	0 ± 0	3 ± 2	7 ± 2 ^{AB}	0 ± 1	3 ± 2	8 ± 2^{AB}
$RPE_{breathing}$*(AU)*	0 ± 0	2 ± 2	7 ± 1 ^{AB}	0 ± 1	2 ± 2	8 ± 2^{AB}

Values are presented as mean ± SD. Heart rate (HR), Blood Lactate $[Lac^-]_B$, minute ventilation ($\dot{V}_E$), oxygen consumption ($\dot{V}O_2$), carbon dioxide production ($\dot{V}CO_2$), Respiratory exchange ratio (RER), Arbitrary units (AU).^{A} = different to baseline $P<0.05$, ^{B} = different to post-LC $P<0.05$.

5.4 DISCUSSION

The aim of this study was to investigate the effects of 6-wks IMT on pre-loaded time-trial performance while carrying a 25 kg thoracic load in a backpack. The novel findings were threefold 1) LC_{TT} performance was improved, 2) despite an increase in respiratory muscle strength post IMT, respiratory muscle fatigue was unchanged relative to pre-intervention values and 3) heart rate and perceived exertion during exercise were attenuated during LC after IMT. Accordingly, this study is the first to demonstrate an ergogenic effect of IMT using a double-blind placebo controlled design during self-paced exercise while carrying a thoracic load.

5.4.1 TIME-TRIAL PERFORMANCE

Performance on baseline LC_{TT} was similar to those described in Chapter 3 and Section 2.10.3..3 Time-trial performance was significantly improved following IMT by 8 ± 4% with no change in PLA. When combined with the significant interaction effect and a very large effect size (d=0.93); these results are regarded as a worthwhile effect (Hopkins, 2000). To date there is no data available to compare this IMT-mediated improvement in load carriage performance. It was previously demonstrated that flow resistive IMT had no effect upon unloaded (i.e., with no backpack) incremental exercise performance in members of the German Special Forces (Sperlich et al., 2009). This study failed to observe increases in P_{Imax} post-IMT and deemed the training intervention ineffective. However, the use of incremental exercise tests to the limit of volitional tolerance as a measure of performance has been widely criticised and known to be unaffected by IMT (Brown et al., 2010; Romer et al., 2002a). Accordingly, the findings here must be compared with data from un-loaded exercise (i.e., no backpack). IMT has repeatedly improved whole-body performance in cycling, running, rowing and repeated sprint exercise with improvements of 3 to 6 % (for a review see Section

1.3.3 and HajGhanbari et al., 2013; Illi et al., 2012). Given the improvement observed here, it has been demonstrated for the first time that IMT has a genuine ergogenic effect upon 25 kg load carriage exercise performance.

5.4.2 Respiratory Muscle Pressure

The mechanism(s) accounting for the improvement in performance are not well understood but are likely explained by improved inspiratory muscle strength and its effect upon cardiorespiratory and/or perception of effort during exercise. Inspiratory muscle strength increased post-IMT (31 ± 26%), similar to previous work (Johnson et al., 2007). Prior to the intervention significant inspiratory muscle fatigue post-LC and LC_{TT} was observed which is consistent with the findings in Chapters 3 and Section 2.10.3.3 using the same protocol and with firefighters exercising with SCBA (Butcher et al., 2006). Post-IMT, P_{Imax} was greater at each time point throughout the experimental trial; however, the magnitude of inspiratory muscle fatigue was similar. This is in contrast to previous studies that show attenuated inspiratory muscle fatigue after cycling time trial exercise (Romer, McConnell, & Jones, 2002a). The findings here are in agreement with Verges et al (2006), who demonstrate similar rates of respiratory muscle fatigue following endurance-focused respiratory muscle training, despite a 272% improvement in respiratory muscle endurance. This was attributed to either 1) an ineffective intervention, this is not the case here as large changes in P_{Imax} with no change in the PLA group were observed, or 2) that IMT failed to attenuate any inspiratory muscle fatigue associated with non-respiratory roles such as supporting posture, spinal stability and the general control of the load upon the thorax. The latter, may explain these findings as wearing a backpack increases postural sway and the reliance upon the diaphragm to stabilise the spine (Hodges, Butler, McKenzie, & Gandevia, 1997). In addition, load carriage fatigues accessory respiratory muscles of the thorax which support pulmonary ventilation during

exercise with high breathing demands (Blacker, Fallowfield, Bilzon, & Willems, 2010). Therefore it is possible that IMT failed to attenuate fatigue that is associated with the non-respiratory functions of the inspiratory muscles, although future research is required to address this. Despite, no reduction in respiratory muscle fatigue after IMT, a significant IMT-mediated change in cardiovascular strain and the perceptions of effort were observed which is attributed to the increase in inspiratory muscle strength.

5.4.3 CARDIOVASCULAR STRAIN

A 7% reduction in HR was observed post-LC after IMT, indicating reduced cardiovascular strain. Reduced cardiovascular strain during loaded breathing tasks has been demonstrated previously after IMT (lowered HR and mean arterial pressure) attributed to increases in strength. IMT facilitates oxidative adaptations within the inspiratory muscles (Witt, Guenette, Rupert, McKenzie, & Sheel, 2007) permitting them to contract at a lower relative intensity (Turner et al., 2012). Here, this was attributed to an attenuated sympathetic efferent response (i.e. reduced metaboreflex activation) to fatiguing inspiratory muscle work whilst at rest. Consequently, after IMT in the present study, reduced cardiovascular strain due to a similar mechanism prior to LC_{TT} may have contributed to the improved time trial performance. The reduction in cardiovascular strain observed in this study may also be explained in part, by normalised intrathoracic pressures swings during the breathing cycle (Miller, Beck, Joyner, Brice, & Johnson, 2002). Load carriage increases gastric pressure which in turn elevates central venous and transdiaphragmatic pressure which in turn alters cardiac pre and after-load, affecting both cardiac output and heart rate (Miller et al., 2002). Following IMT P_{Emax} was increased which may also permit the expiratory muscles to operate at a lower relative intensity during exercise facilitating venous return (Butcher et al., 2006) and normalising this cardiovascular response.

5.4.4 PERCEPTUAL RESPONSES

A ~4% reduction in whole body discomfort and a ~10% reduction in leg discomfort was observed during LC following IMT; similar to previous observations during rowing and cycling time trial exercise (Romer et al. 2002a; Volianitis et al. 2001) and isolated breathing challenges (Verges et al, 2007). In the present study, these reductions were independent of exercise intensity and ventilatory demand since the intensity was fixed during LC and produced a similar ventilatory response (Tables 5.2 and 5.3). IMT has been shown to reduce the oxygen cost of breathing for a given pulmonary ventilation (Turner et al., 2012). Therefore, repeated bouts of IMT may alter the discharge frequency of mechano-sensitive type III and IV nerve afferents due to lower metabolic demand, reducing afferent feedback and the perception of breathing discomfort (Sinoway et al. 1996). It is possible that the reduction in perception of effort is linked to the attenuation of the metaboreflex whereby limb blood flow is sustained and limb fatigue reduced since IMT increases the threshold for activation of this response (McConnell & Lomax, 2006). Recent evidence suggests that limb discomfort induced by fatiguing knee extensor exercise heightens the perception of breathing discomfort with no change in pulmonary ventilation (Grippo et al., 2010; Sharma, Morris, & Adams, 2015). Therefore, improved locomotor muscle function following IMT due to improved limb blood flow (attenuated metaboreflex) would attenuate limb discomfort and also subsequently breathing discomfort. Despite this notion, during sub-maximal exercise (LC: 59% $\dot{V}O_2$ peak) any reductions in limb blood flow are likely to be compensated by increased locomotor muscle oxygen extraction (Jones et al., 2011; Romer, Haverkamp, Lovering, Pegelow, & Dempsey, 2006). IMT-mediated reductions in breathing discomfort are correlated with improved exercise tolerance (Romer, McConnell, & Jones, 2002b) therefore regardless of the mechanism(s) afferent discharge, whether originating within the locomotor and/or respiratory musculature are incredibly important, as they both project to the

sensorimotor cortex which regulates central motor drive (Amann & Dempsey, 2008). Accordingly a reduction in afferent feedback from these sources would improve central motor drive for the subsequent time trial effort.

5.5 CONCLUSION

Inspiratory muscle training improved 2.4 km time trial performance when exercise was performed with 25 kg thoracic load carriage and pre-loaded with 60 min sub-maximal exercise. These findings are most likely explained by an increase in inspiratory and expiratory muscle strength and the subsequent effects upon cardiovascular strain and the sensations of the perception of effort. These findings may have implications for both occupational and recreational groups where load carriage is a critical role-related task. Importantly, inspiratory muscle fatigue was unaffected after IMT. Consequently, IMT may have failed to attenuate any inspiratory muscle fatigue associated with non-respiratory roles such as supporting posture and spinal stability. Therefore, employing a mode of IMT which also targets these muscles may attenuate respiratory muscle fatigue and further improve performance. Such techniques have recently come to light and are known as functional IMT (Tong, McConnell, et al., 2014). The effects of functional IMT techniques upon respiratory muscle fatigue and load carriage performance are not yet known and subsequently are the aim of the final study of this thesis.

CHAPTER 6

FUNCTIONAL IMT IMPROVES LOAD CARRIAGE PERFORMANCE GREATER THAN TRADITIONAL IMT TECHNIQUES

6.1 INTRODUCTION

Chapters 3, 4 and 5 demonstrated the physiological consequences of exercise while carrying heavy external loads upon the thorax and the negative effects this has upon P_{Imax} and whole body performance. Accordingly, investigations of acute and chronic inspiratory muscle loading were conducted demonstrating an ergogenic effect of the latter and not the former. Acute inspiratory loading was ineffective in eliciting an improvement in performance on LC_{TT} despite transient increases in P_{Imax} (Chapter 4), but six weeks of chronic loading increased baseline levels of P_{Imax} to a greater extent and provided an ergogenic effect compared with a sham placebo (Chapter 5). Although IMT significantly elevated P_{Imax} at each time point during LC and LC_{TT} post IMT, it did not attenuate inspiratory muscle fatigue; therefore, the performance enhancement may have been restricted.

It was hypothesised in the previous chapter that IMT methods used do not target the accessory musculature, which are recruited extensively throughout load carriage tasks and serve numerous non-respiratory related roles. The use of chronic loading improved exercise performance on a 2.4 km self-paced time trial; however, it failed to attenuate inspiratory muscle fatigue associated with non-respiratory roles such as supporting posture and spinal stability. Recently it has been suggested that functional inspiratory muscle training (IMT_F) may elicit positive performance adaptations above that of traditional IMT alone for running exercise (Tong, McConnell, et al., 2014). IMT_F targets the inspiratory muscles throughout the length-tension range adopted during exercise tasks as IMT is performed during body positions adopted during those reflective of performance. This form of training provides a stimulus to both the inspiratory musculature of the thorax and the core stability muscles of the abdomen facilitating both respiration and spinal stability during exercise tasks (Boussana et al., 2003; Tong & Fu, 2006). This is important in load carriage activities as recruitment of the

respiratory muscles is exacerbated with thoracic loads to provide greater spinal stability (Heller et al., 2009; Hodges et al., 2001) as anterior trunk displacement increases lumbosacral forces (Goh, Thambyah, & Bose, 1998). Importantly, the ventilatory requirements of the inspiratory muscles are prioritised over stabilisation of the spine during exercise with a high breathing demand, which can lead to impaired spinal and postural stability during exercise (Hodges et al., 2001; Janssens, Brumagne, Polspoel, Troosters, & McConnell, 2010).

Submaximal walking with thoracic loads also induces neuromuscular impairment and reduces locomotor force during isometric knee extension tasks (Blacker, Fallowfield, Bilzon, et al., 2013). This may be exacerbated during high intensity exercise as assessed throughout this thesis in the form of a load carriage time-trial. In Chapter 3, it was suggested that inspiratory muscle fatigue may exacerbate locomotor muscle fatigue and impair whole body performance via a sympathetically-mediated redistribution of blood flow away from the limbs, presumably in favour of the inspiratory musculature. In Chapter 5, inspiratory muscle fatigue was not attenuated post-IMT presumably because increased inspiratory muscle work that is associated with maintaining spinal stability is not targeted by IMT. Therefore, if this was the case as it is with the use of IMT_F methods, it is attractive to speculate that limb muscle force would be sustained. The primary mechanism here being that the distribution of $\dot{Q}$ would be normalised through an attenuation inspiratory muscle fatigue; thus reducing the work required by the inspiratory and core musculature, thus preserving spinal stability.

IMT_F may have a magnified effect, relative to traditional IMT, upon load carriage performance because of the dual role of the inspiratory muscles, such as sustaining ventilation and supporting spinal stability and posture, during exercise with load carriage. This is likely to be achieved by reducing inspiratory and locomotor muscle fatigue and facilitating the inspiratory muscle contribution to spinal stability through activation of other synergistic core

respiratory muscles. Accordingly, the aims of the current study were to i) confirm the ergogenic effect of traditional IMT upon load carriage time trial performance, ii) investigate the ergogenic effect of IMT_F compared to traditional IMT, and iii) identify whether IMT_F attenuates inspiratory muscle and locomotor muscle fatigue compared to traditional IMT. It was hypothesised that IMT_F would improve performance above that of traditional IMT and attenuate locomotor and respiratory muscle fatigue.

6.2 METHODS

6.2.1 PARTICIPANTS

Following ethics approval from the host university, 17 non-smoking healthy males with previous experience of load carriage through regular recreational load carriage activities, and/or prior involvement in previous experimental chapters were recruited to this study (Table 6.1). The participants were pooled for phase 1 of the study (4 wks traditional IMT) and randomly split, matched for P_{Imax} for phase 2 of the study (4 wks IMT_F or 4 wks traditional maintenance IMT hereon referred to as IMT_{CON}). Prior to the study, participants were fully briefed on all procedures as outlined in Section 2.1. Initially 20 participants were recruited to the study; however, three withdrew due to personal reasons during the study.

Table 6.1 Mean ±SD for the descriptive characteristics of the participants during both phases of the study (n=17).

	Phase 1:	**Phase 2:**	
	Pooled data (n=17)	**IMT_F (*n* = 9)**	**IMT_{CON} (*n* = 8)**
Age (years)	23.4 ± 5.6	22.4 ± 2.3	24.7 ± 8.6
Body Mass (kg)	75.4 ± 11.7	73.3 ± 10.3	80.2 ± 13.6
Height (m)	1.77 ± 0.06	1.78 ± 0.03	1.77 ± 0.09
P_{Imax}(cmH_2O)	151 ± 36	147 ± 35	156 ± 39
P_{Emax} (cmH_2O)	134 ± 54	127 ± 30	147 ± 74
FEV_1 (L)	3.86 ± 0.86	3.95 ± 0.35	4.15 ± 0.58
FVC (L)	4.75 ± 0.49	4.91 ± 0.61	4.75 ± 0.49
FEV_1/ FVC (%)	85 ± 9	80 ± 12	83 ± 11
PEF ($L \cdot min^{-1}$)	545 ± 74	561 ± 112	552 ± 89
Body Fat (%)	18.7 ± 5.3	17.4 ± 5.8	20.2 ± 4.4
Lean body mass (kg)	58.3 ± 6.9	57.6 ± 5.5	60.9 ± 8.1
Fat mass (kg)	14.0 ± 5.6	12.6 ± 5.9	16.0 ± 5.3
Bone mineral density (g/cm^2)	1.32 ± 0.11	1.32 ± 0.10	1.34 ± 0.13
Bone mineral content (kg)	3.29 ± 0.49	3.28 ± 0.40	3.44 ± 0.60
$\dot{V}O_2$ peak ($L \cdot min^{-1}$)	3.61 ± 0.45	3.53 ± 0.43	3.82 ± 0.43
$\dot{V}O_2$ peak ($ml \cdot kg^{-1} \cdot min^{-1}$)	47.81 ± 7.08	46.63 ± 6.87	48.73 ± 8.32
Whole Body Peak Force (N)	3156.0 ± 556.5	3113.8 ± 304.4	3287.8 ± 546.2
Isometric Knee Extensor Peak Force (N)	561 ± 116	569.8 ± 78.4	587.0 ± 129.6
Training Adherence (%)	94 ± 7	94 ± 5	94 ± 9

Values are presented as mean ± SD. P_{Imax}, maximal inspiratory pressure, P_{Emax}, maximal expiratory pressure, FVC, forced vital capacity, FEV1, forced expired volume in one second, PEF, peak expiratory flow; $\dot{V}O_2$ peak; maximal oxygen uptake in absolute units and relative to body mass.

6.2.2 PRELIMINARY ASSESSMENTS

Prior to experimental trials, participants completed two preliminary assessments; the first consisted a whole body assessment of body composition as described in Section 2.7.1, followed immediately by an assessment of whole-body maximal strength as described in full elsewhere (Kawamori et al., 2006), in order to determine a potential relationship between levels of maximal strength and performance on the load carriage time trial and volitional measures of strength. Briefly, participants completed a warm up cycling at 70 RPM and 105 W for 10 mins before performing 6 mid-thigh isometric pulls (Kawamori et al., 2006). A weightlifting barbell (Elieko, 2XL, Pullum Sports, Luton, UK) was positioned mid-thigh across a heavy duty power rack (Pullum Sports, Luton, UK) and loaded with calibrated competition weights ranging from 25kg to 10kg (Elieko, Pullum Sports, Luton, UK) to a total weight of 270 kg to ensure the bar was immovable during efforts (Figure 6.1). Additional safety controls were put in place, which included two spotters in the transverse plane and one in the sagittal plane, the role here was to ensure displacement of the bar did not occur and also to ensure correct form was maintained throughout each effort. A force platform (Fitness Technology, 400 series, Skye, Australia) was positioned centrally underneath the bar operated via a laptop computer (Toshiba, TECRA A1016E, Surrey, UK) using the Ballistic Measurement System interface (Fitness Technology, Skye, Australia). The force platform was calibrated prior to each test to determine zero force and a force of known value (250 KG; 2500.69, Newtons (N). Once calibrated, the height of the barbell was individually adjusted to ensure it was positioned in the centre of the thigh for each participant. Weight lifting straps (Rubicon Sports, Hampshire, UK) were worn at the wrist by all participants during each effort to maximise grip strength. Participants completed 3 practice attempts before completing 3 experimental efforts and strived to pull the bar vertically for 3 s. Strong verbal encouragement was given throughout. Attempts were separated by 5 min and used to determine: Peak force

(N), peak power (Watts), max rate of force development ($N \cdot s^{-1}$), time to peak power (s) and time to peak force (s). The maximum value of peak force and corresponding values were used for analysis. On the second visit, participants completed a maximal incremental exercise test to volitional tolerance to determine $\dot{V}O_2$ peak, as described in Section 2.10.1.

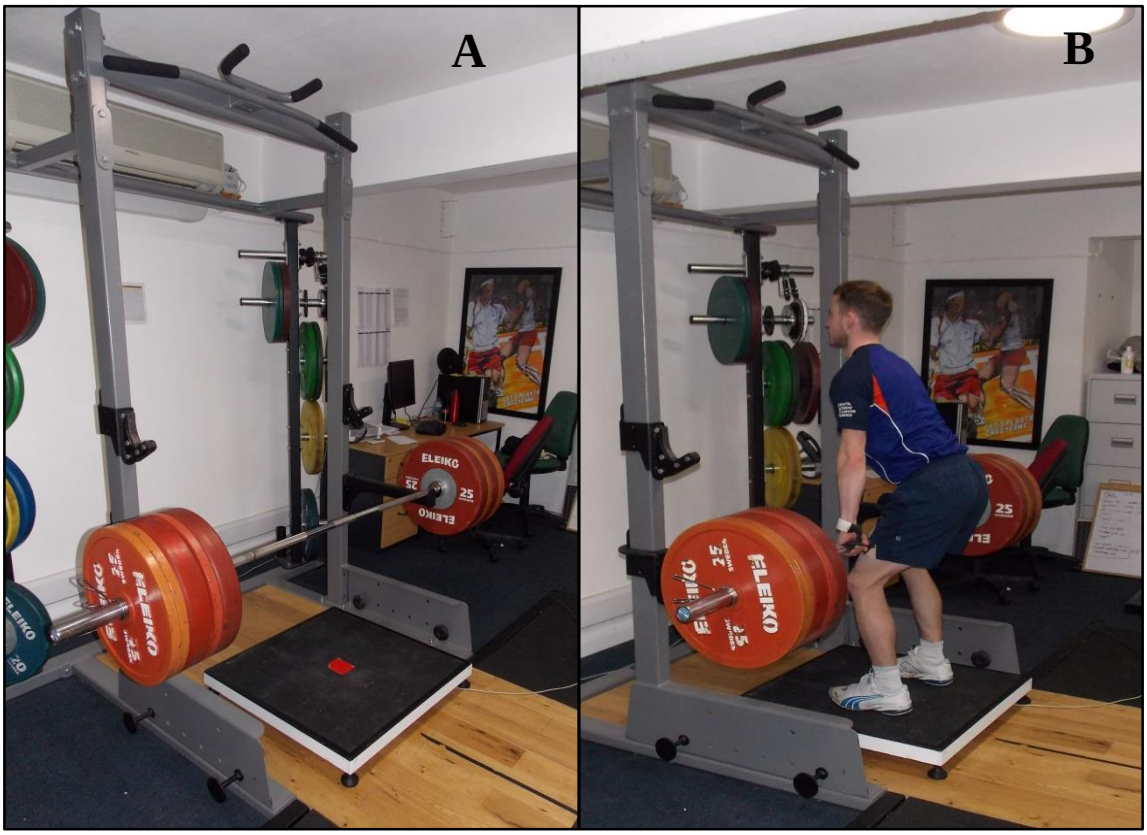

Figure 6.1 Mid-thigh isometric pull used to assess maximal strength. Panel A shows the set up adopted and Panel B shows a participant completing a manoeuvre.

6.2.3 EXPERIMENTAL TRIAL

Participants completed the experimental protocols on three occasions (at baseline, post-4 wks and post 8 wks); each separated by a minimum of four weeks (8 week total duration of study). Due to the length of time between each experimental trial, participants repeated the shortened familiarisation session one week prior to the post-4 wk and post-8 wk experimental trials. Resting measures were conducted prior to exercise as discussed

previously and included: volitional mouth pressures (Section 2.3), pulmonary function (Section 2.4), measurements of blood $[Lac^-]_B$ and $[glucose^-]_B$ (Section 2.6.1), pulmonary ventilation (Section 2.5.2), heart rate (Section 2.8.1), whole body perceptual responses and specific to legs and breathing discomfort (Section 2.8.2), and were repeated immediately post LC and post LC_{TT}. Measures of volitional mouth pressures and pulmonary function differed to those stated in previous chapters as attempts were performed seated on a chair (Figure 6.2), compared with standing as adopted in previous chapters due to simultaneous measures of voluntary and electrically evoked stimulation of the quadriceps muscle group (m. quadriceps), described below.

Participants completed a sport-specific endurance plank test (SEPT) in all trials and prior to all resting (from here referring to all pre LC measures) physiological measures with the exception of $[lac^-]_B$ and HR. Participants completed the sport-specific endurance plank test (SEPT) described previously within literature (Tong, McConnell, et al., 2014) and is a valid and reliable assessment tool of core strength (Tong, Wu, & Nie, 2014). Participants were asked to maintain a prone bridge throughout the test with maximum effort and correct form throughout the following stages with no rest between transitions: (1) hold the basic prone bridge position for 60 s, (2) lift the right arm off the ground and hold for 15 s, (3) return the right arm to the ground and lift the left arm for 15 s, (4) return the left arm to the ground and lift the right leg for 15 s, (5) return the right leg to the ground and lift the left leg for 15 s; (6) lift both the left leg and right arm from the ground and hold for 15 s, (7) return the left leg and right arm to the ground, and lift both the right leg and left arm off the ground for15 s; (8) return to the prone bridge position for 30 s, (9), and then repeat all stages from (1) to (9) until the maintenance of the prone bridge failed. The test was terminated when the hip failed to be maintained at the required level after receiving two warnings and the time to the limit of tolerance provided an index of global core muscle function. On completion of this,

participants were seated on the chair used to assess m. quadriceps, whilst the remaining resting measures were conducted.

Figure 6.2 Custom built adjustable chair used during all isometric and electrically evoked procedures that contains the horizontal back bar in which the strain gauge is attached.

6.2.3.1 VOLUNTARY AND ELECTRICAL STIMULATION

Volitional and non-volitional techniques were used to assess maximal force output from the m. quadriceps and were conducted at rest; post LC, post LC_{TT} and 30 min post exercise ($post_{30min}$) for each experimental trial. For all tests, participants were secured to the custom built chair. The configuration of the chair was individually adjusted to ensure the hip and knee were at 90° flexion, with measures recorded using a handheld goniometer (Cranlea, Birmingham, UK) and used for all subsequent trials. Velcro straps were placed around the participants' chest and waist to restrict movement of the upper body and hips. A cuff was placed around the participants' ankle (proximal to the fibular notch and medial malleolus) and

attached to an s-type load cell (Interface, SM-1000, Arizona, USA) capable of withstanding 453.59 KG-force. The load cell was calibrated prior to use with calibrated weights ranging from 0 kg – 50 kg, at 2.5 kg increments. The force (N) exerted by the weights was positively correlated (r = 0.999) with the force output of the strain gauge and had a mean bias of -0.14 N (Figure 6.3. and 6.4).

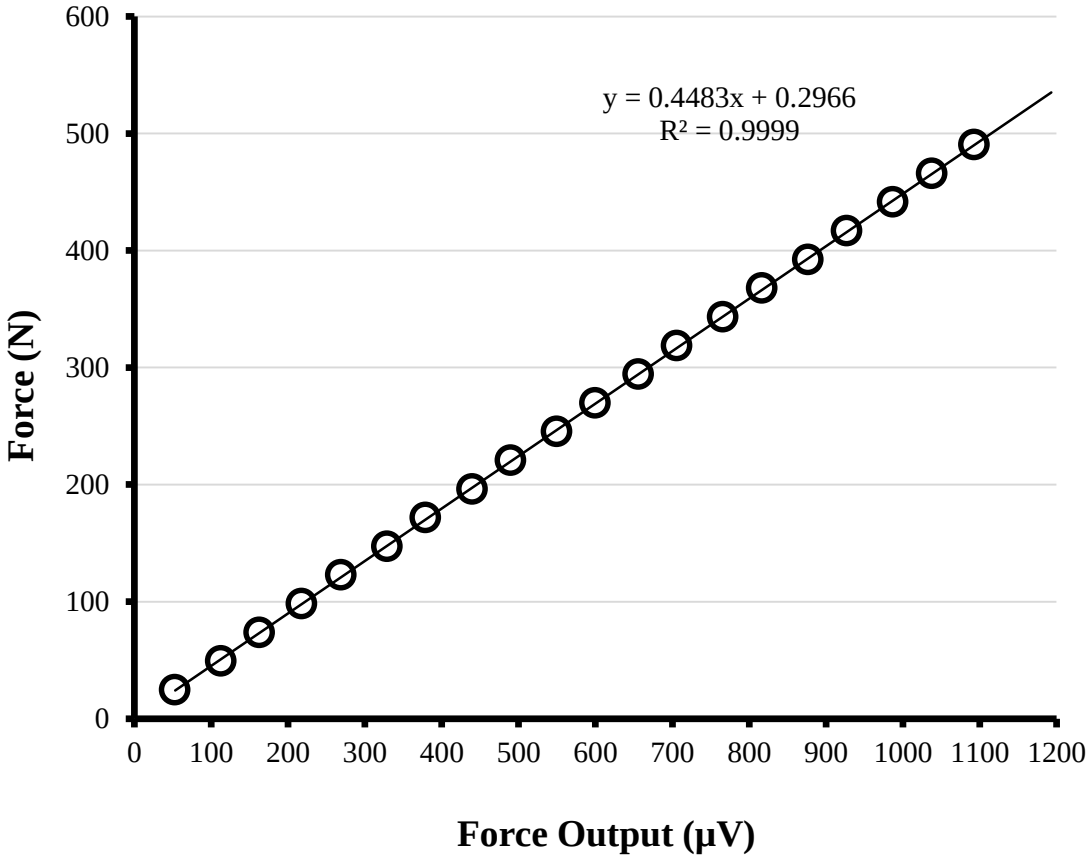

Figure 6.3 Calibration of the load cell using calibrated weights ranging from 0-50 kg. The known force exerted by the disks (N) was correlated against the force values derived from the software demonstrating a near perfect relationship (r = 0.999).

The load cell was connected to the base of the chair using carabiners as shown in Figure 6.5. The force produced from the m. quadriceps femoris was amplified (CED, 1902, Cambridge, UK) and converted to a digital signal (CED, Micro1401, Cambridge, UK) and recorded on a computer at 1000 Hz (CED, Spike 2 Version 8, Cambridge, UK).

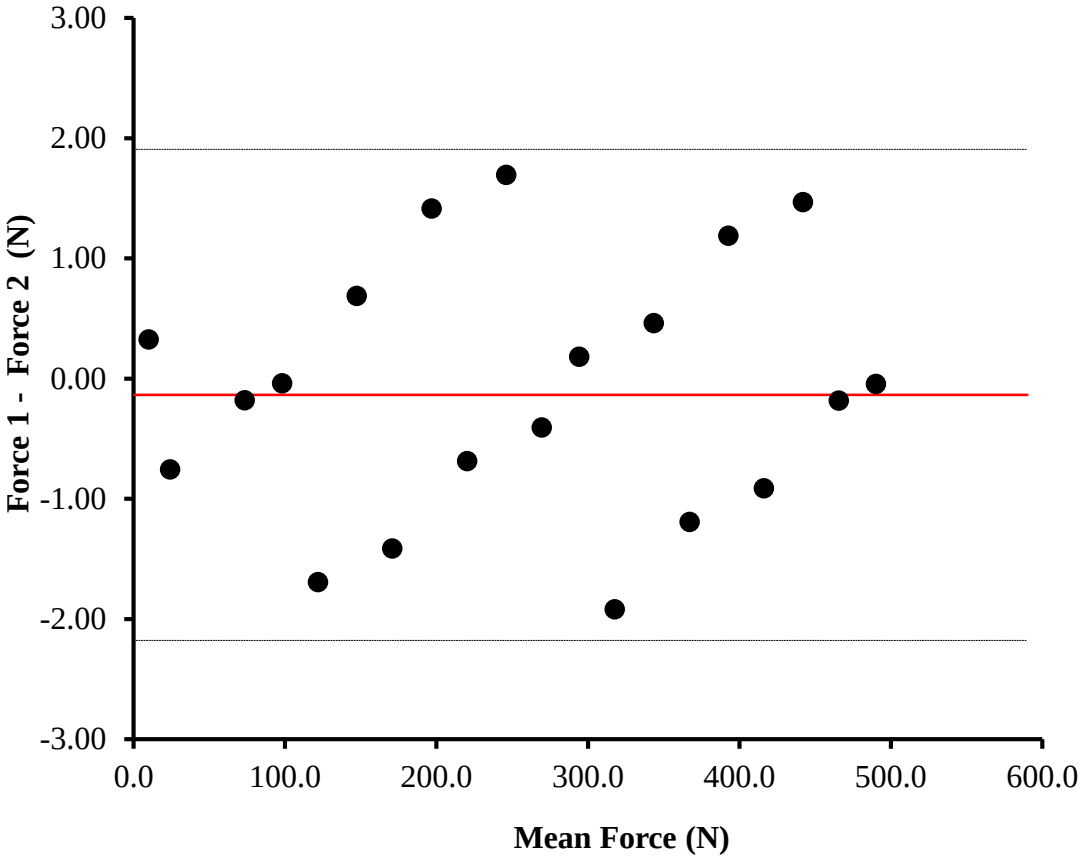

Figure 6.4 Bland Altman plot for the calibration data of the load cell used for this study, all data points within ± 1.96 of the mean. Force 1 is the value of the known calibrated weights and force 2, the value obtained from the load cell.

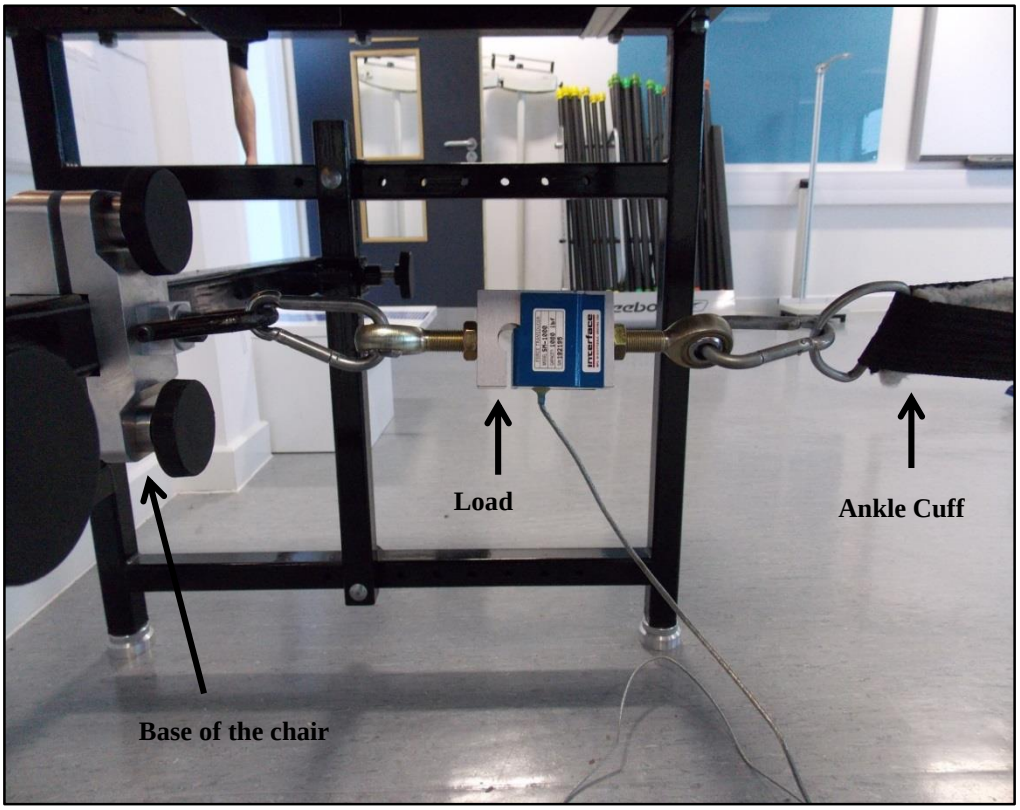

Figure 6.5 Connection of the load cell via carabiners to the custom built chair used during all isometric and electrically evoked procedures.

Electrical stimulation was delivered through an electrical muscle stimulator (Model DS7A, Digitimer Limited, Welwyn Garden City, UK); pulse frequency and duration were controlled via the Micro1401 (Cambridge Electrical Design, 1401, Cambridge, UK). Two rubber surface electrodes (12 cm x 8 cm) were contained within a damp sponge pad and placed proximal to the patella and over the muscle belly of the m. quadriceps femoris in the proximal third part of the thigh of the non-dominant leg (Figure 6.6). The electrodes were held in place with a tubigrip compression bandage and the position of the electrodes was marked using indelible ink to ensure accurate placement on subsequent trials. The order of the technical measures used here are shown in Figure 6.7 and have been discussed previously (Blacker, Fallowfield, et al., 2010; Blacker, Fallowfield, & Willems, 2013) and are detailed briefly below. A typical example of the force recorded during an electrically evoked twitch is illustrated in Figure 6.8.

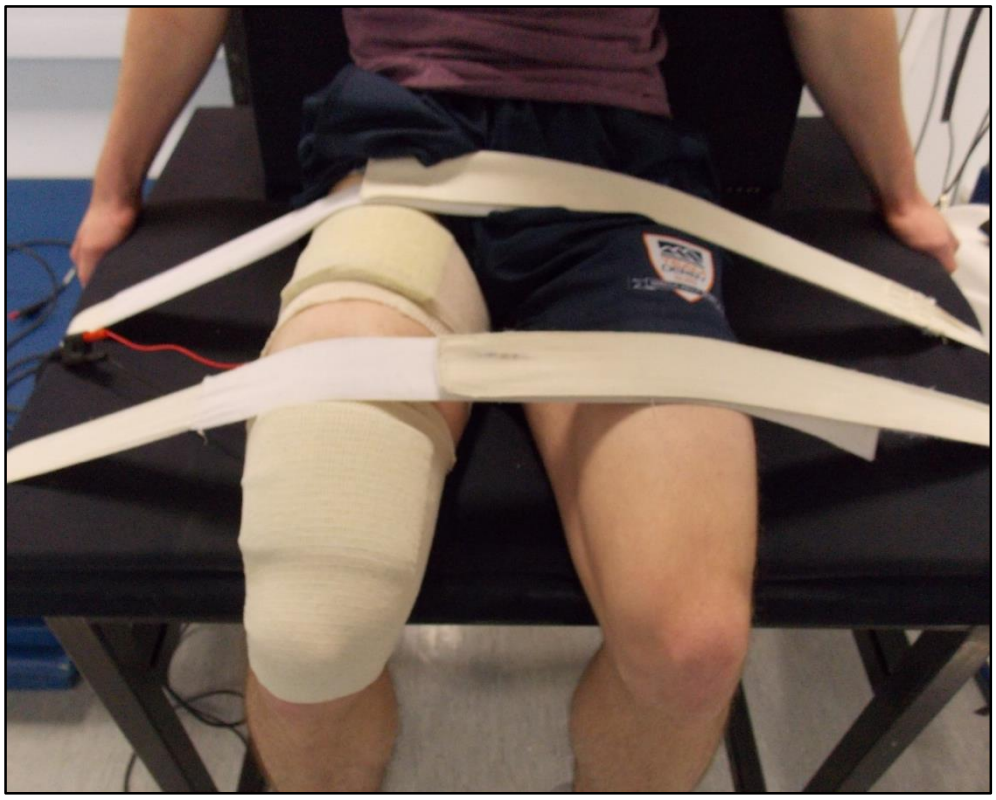

Figure 6.6 Electrode placement on the m. quadriceps muscle group using during all electrically evoked procedures.

6.2.3.2 VOLITIONAL MEASURES OF M. QUADRICEP FORCE

Participants produced a 3 to 5 s maximal isometric voluntary contraction (MVC) of the m. quadriceps with strong verbal encouragement. This was used to test the force capability of the m.quadricep and provides information regarding the ability to voluntary produce maximal muscle force (Blacker, Fallowfield, & Willems, 2013). If it was deemed through observation of the data that the effort was not maximal the procedure was repeated following 2 min rest; this occurred only on a few instances during the study. The single absolute highest value and the highest mean force values over a 0.25 and 0.50 s time period during contractions were calculated. A typical example of the force recorded during a MVC is illustrated in Figure 6.8, panel B.

6.2.3.3 NON-VOLITIONAL MEASURES OF M. QUADRICEP FORCE

The methods adopted to calculate the output intensity of the muscle stimulator to ensure maximal muscle fibre recruitment varies within the literature. Studies use either 1) a pre-determined stimulation intensity determined upon first visit which is fixed for all subsequent visits (Blacker, Fallowfield, & Willems, 2013) or 2) an output intensity determined separately upon each visit (Romer et al., 2006). In this thesis, the electrical current delivered from the stimulator was calculated upon the first visit to the lab and remained fixed for all future stimulations in all experimental trials. This method was chosen as it provides the greatest within and between session reliability which was determined during pilot work. Briefly, 10 participants completed four trials (baseline, post LC, post LC_{TT} and $post_{30min}$) within a single day and repeated this protocol over three days separated by 24 h using method 1 (see Appendix 4, Table 1 for detail) and during a different week using method 2 (see Appendix 4, Table 2 for detail). Method 1 had far superior between and within day reliability and the results of this work are clearly demonstrated in full within the appendices. The electrical stimulation protocol contained a single twitch, doublet, 20 Hz and 50 Hz stimulation, which are described below.

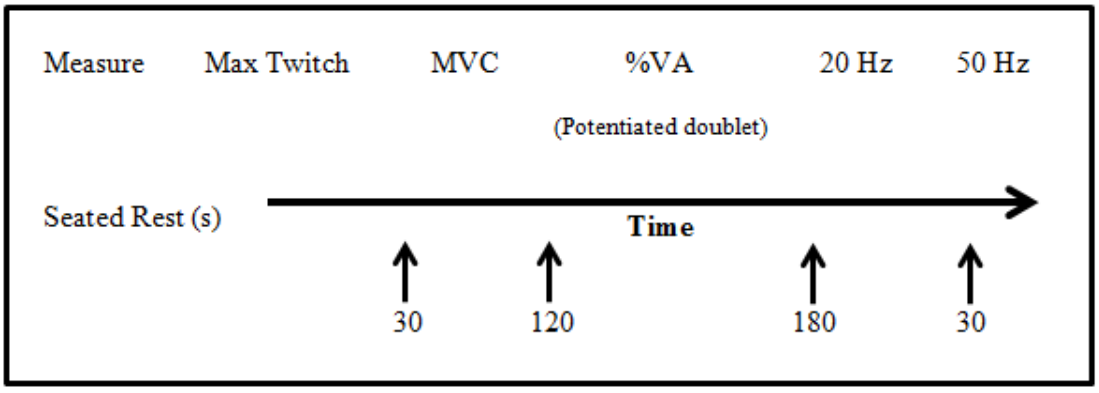

Figure 6.7 Test order for isometric contractions of the m. quadriceps femoris muscles using voluntary activation and surface electrical stimulation. Figure adapted from (Blacker, Fallowfield, & Willems, 2013).

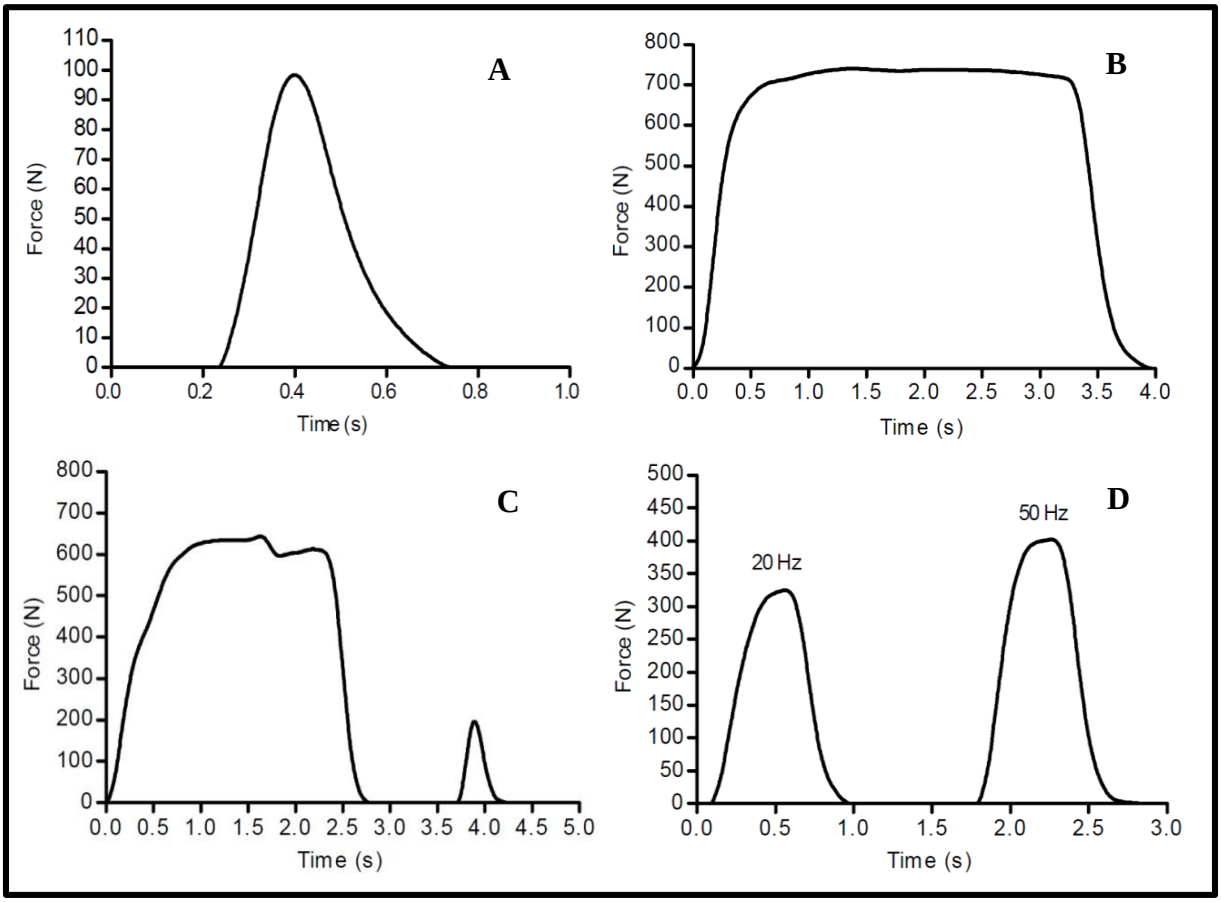

Figure 6.8 Typical responses recorded during isometric contractions of the m. quadriceps femoris (knee and hip angle 90^{o}) during voluntary and electrically stimulated contractions: (A) maximal voluntary contraction, (B) resting twitch, (C) potentiated doublet and (D) 20 Hz and 50 Hz stimulation. Figure edited from Blacker et al., (2013).

6.2.3.3.1 ELECTRICALLY EVOKED TWITCH

Electrically evoked twitches are non-volitional methods, used to determine muscular response to a single maximal stimulation and can be used to describe changes in force generating capacity of the muscle (Blacker, Fallowfield, & Willems, 2013). For all measures, participants were instructed to relax their leg and provide minimal tension in the ankle cuff to ensure that there was no slack in the connection to the load cell (<22.5 µV or <10 N). A single, 50 µs pulse at pre-determined stimulation current was applied to the m. quadriceps femoris (group mean current 308.2 ± 54.8 mA). The maximal twitch was recorded and for the following parameters measured: (a) peak force (N), the maximal force value of the twitch, (b)

contraction time (s), (c) average rate of force development ($N \cdot s^{-1}$), peak force / contraction time; (d) half relaxation time (s); (e) maximal rate of force development ($N \cdot s^{-1}$), and (f) maximal rate of force decrease ($N \cdot s^{-1}$).

6.2.3.3.2 INTERPOLATED DOUBLET

The interpolated doublet sees an electrical stimulus applies to volitional contractions (MVC) which provide an indication of the origin of fatigue. Participants produced an MVC (as described above). A doublet pulse (two maximal single twitches separated by 10 ms) was applied to the m. quadriceps femoris during the plateau phase of the MVC, and once, immediately after the MVC when participants returned to rest (potentiated doublet). The firing of the second pulse (at rest) was automated when the force exerted on the load cell was <10 N. A typical example of the force recorded during a MVC with an interpolated and potentiated doublet is illustrated in Figure 6.8, panel C. Consequently, percent voluntary activation (%VA) was calculated using the following equation:

$$\%VA = 100 - (\text{Superimposed MVC Doublet force} / \text{Post MVC Doublet force}) \times 100$$

6.2.3.3.3 20HZ AND 50HZ STIMULATION

Stimulating the muscle with a tetani of stimuli (0.5-2.0 s) at low (10-20 Hz) and high 50-100 Hz) frequencies and expressed as a ratio, detects the presence of high and/or low frequency fatigue following exercise (Blacker, Fallowfield, & Willems, 2013). Continuous 20 Hz (intra-stimulation duration: 0.025 s) and 50 Hz (intra-stimulation duration: 0.01 s) stimulations (total stimulation for both 0.5 s), with 30 s rest between stimulations, were applied to the m. quadriceps femoris using a sub-maximal twitch current (50% of max stimulation amplitude). Sub-maximal currents were preferred as it has been consistently shown to provide reliable estimates of contractile properties (Edwards et al., 1977) and is

more tolerable for participants. The ratio of the forces recorded at 20 Hz and 50 Hz was calculated, reductions in this ratio indicates the presence of low frequency fatigue (LFF) and an increase in this ratio indicates high frequency fatigue (HFF; Jones, 1996). The parameters outlined for the resting electrically evoked twitch were calculated for the 20 Hz and 50 Hz stimulations. A typical example of the force recorded during 20 and 50 Hz stimulations is illustrated in Figure 6.8, panel D.

6.2.4 TRAINING INTERVENTION

All participants completed two experimental trials either side of a 4 wk IMT intervention that consisted of thirty dynamic inspiratory efforts were performed twice daily against a pressure-threshold load of 50% P_{Imax}. Each inspiratory manoeuvre was initiated from residual volume and subjects strove to maximise V_T. This was a shorter protocol than Chapter 5 but is effective in eliciting an adaptive response to the chest wall inspiratory muscle and the diaphragm (Brown et al., 2014) and an ergogenic effect (Tong, McConnell, et al., 2014). Following this, participants were randomly assigned to either a 4 wk traditional maintenance IMT intervention (IMT_{CON}) or 4 wk functional IMT intervention (IMT_F,) after which they completed a final experimental trial. Both groups were matched for P_{Imax} and there were no between or within group differences in any descriptive characteristic or baseline measurement (P>0.05).

6.2.4.1 FUNCTIONAL IMT (IMT_F)

IMT_F comprised three training sessions per week and required the participant to complete four inspiratory loaded core muscle training exercises per session. One session per week was conducted in the laboratory to monitor progress and technique and the remaining two were conducted at home. For each inspiratory muscle effort participants were instructed to inhale forcefully through the device as they initiated the required body actions (detailed

below) from the starting position, and exhaled slowly when returning to the starting position. The exercise volume for IMT_F was progressive across the four weeks and began with two sets of 10 repetitions (consisting of one inspiration and expiration) in each set in the first week and the repetitions were increased to 14 during week 2. In weeks 3 and 4 the number of sets increased to three and the repetitions in each set were increased progressively from 14 to 18 (McConnell, 2011; Tong, McConnell, et al., 2014). The total exercise volume during IMT_F was 1548 training breathes

The exercises (Figure 6.9) completed during IMT_F were specific to running (McConnell, 2011; Tong, McConnell, et al., 2014). Briefly they included: raised alternating crunches (Figure 2.11, Panel C) whilst the "V" position was maintained and with hips flexed on the floor both hands were positioned behind the head, rotating the trunk alternatively with elbow towards the opposed bended knee. After returning to the starting position this was completed on the opposite side. Swiss ball crunches (Figure 2.11, Panel B); participants maintain the press up position with ankles resting on a Swiss ball, the pelvis was then raised upwards from the straight body line and the knees were bent before returning to starting position. Prone bridge (Figure 2.11, Panel A): participants lay on their back with their body weight on their heels to maintain a straight body line, then whilst bracing the abdominal muscles participants alternately raised the straightened left leg as high as possible before returning to rest and subsequently raising the right leg. Finally, dynamic bird dog (Figure 2.11, Panel D): the left hand and right leg were raised from a plank position to extend the arm and leg until both were horizontal, then returned to the starting position where the alternate arm and leg were raised.

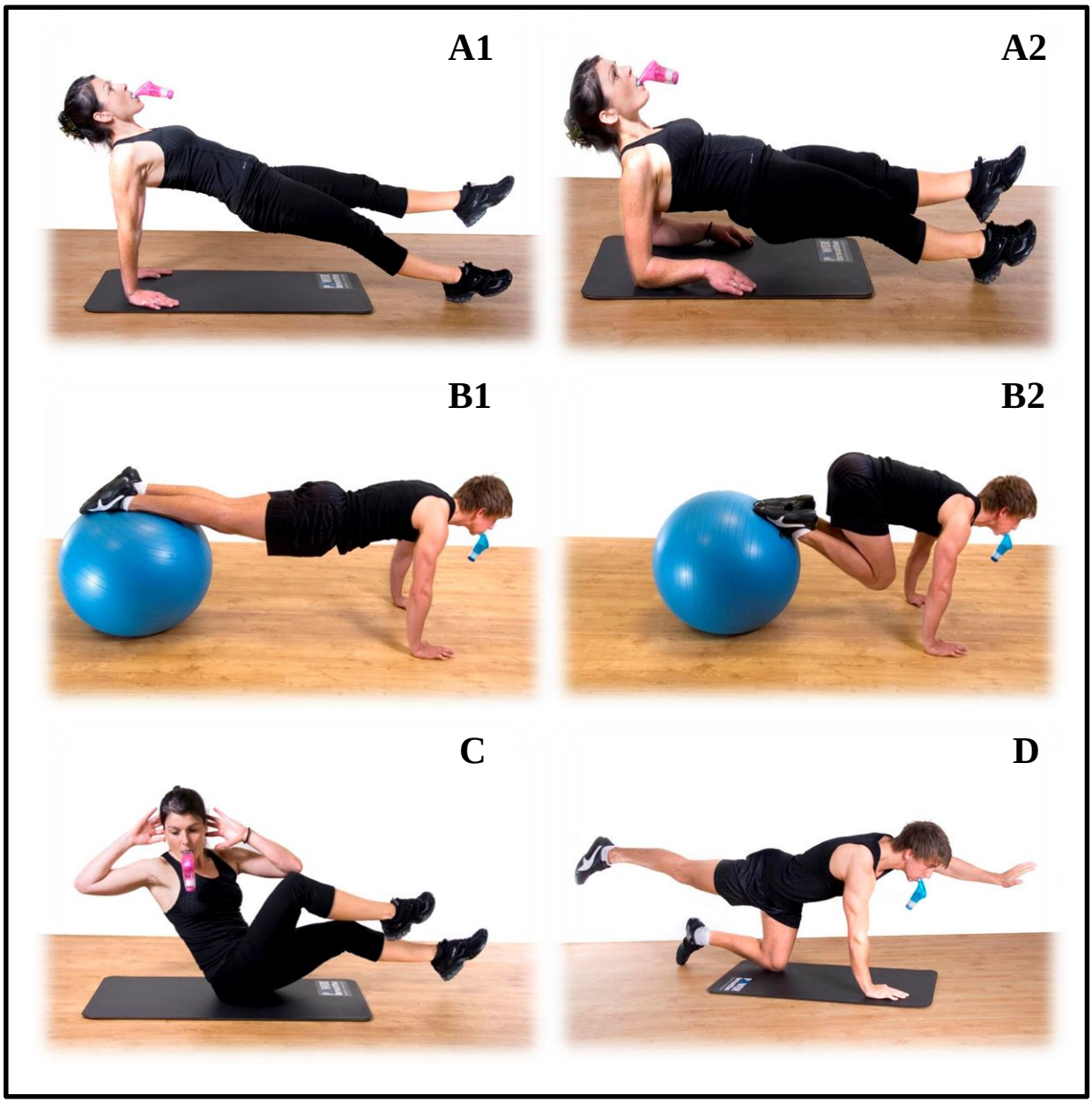

Figure 6.9 Schematic of the four core muscle exercises that were combined with IMT and completed by the IMT_F group, A) bridge, B) swiss ball squat thrusts, C) raised alternating crunch, D) dynamic bird dog. All images Printed with author's permission (McConnell, 2011).

6.2.4.2. CONTROL GROUP (IMT_{CON})

The control group (IMT_{CON}), completed a maintenance intervention which consisted of 30 inspiratory efforts at ~50% P_{Imax}, once daily 3 times per week for 4 wks which has been shown to preserve P_{Imax} (Romer & McConnell, 2004b). During each phase of the intervention the device was calibrated bi-weekly to ensure that training intensity was maintained at 50% P_{Imax}.

6.2.5 STATISTICAL ANALYSIS

The reliability of determining current for subsequent electrical stimulation trials was assessed within session (four time points over three days) across all 12 time points and across all baseline measures on each day. Normal distribution was tested and confirmed using a Kolmogorov–Smirnov test; data was then examined for heteroscedasticity (i.e. a positive relationship between measurement differences) using Pearson's product moment correlation coefficient). Heteroscedasticity was present within the data; therefore, log ratio LoA were calculated for each variable and used for analysis (Atkinson & Nevill, 1998). One-way repeated measures ANOVA were used to examine systematic differences over time for each variable, statistical significance was set at $P< 0.05$ for all tests. The mean square error (MSE) value from the ANOVA was used to determine the 95% LoA (Atkinson & Nevill, 1998) using the following equation:

$$95\% \text{ LoA} = 1.96 \times \sqrt{2} \times \text{MSE}$$

Within group differences and interaction effects for physiological and performance dependent variables were assessed using a 3 (time point: rest, post-LC, post-LC_{TT}) x 3 (LC Trial baseline vs. post-4 wk vs. post-8 wk) measures ANOVA with Tukey's post hoc analysis. The variables derived from the electrical stimulation measures were analysed using a 4 (time point: rest, post-LC, post-LC_{TT}, $Post_{30min}$) x 3 (LC Trial baseline vs. post-4 wk vs. post-8 wk)

ANOVA. All analysis was conducted using SPSS for Windows (Chicago, IL, USA). Between group differences (IMT_F vs. IMT_{CON}) were assessed using independent samples T-Tests. For all statistical tests, a priori α was set at 0.05 and all results are presented as mean ± SD. Effect sizes were calculated using Cohen's *d* ($d=(x^1–x^2)$/pooled σ), judgements were made on the magnitude of the observed effect based on the 'minimal worthwhile effect' as described in previous literature (Hopkins, 2000).

6.3 RESULTS

6.3.1 TIME TRIAL PERFORMANCE

Pre-intervention baseline time trial performance was 15.93 ± 2.30 min with a mean speed of 9.5 ± 1.5 $km \cdot h^{-1}$. For phase 1, post 4 week IMT LC_{TT} performance improved 1.19 ± 0.83 min *(*$P<0.01$, effect size: d=0.56, Figure 6.10) as did mean speed (0.7 $km \cdot h^{-1}$, 7.5%). For phase 2, participants were randomly assigned to either IMT_F or IMT_{CON} with groups matched for LC_{TT} time (IMT_F, 14.11 ± 2.14 min and IMT_{CON} 14.75 ± 1.74 min; $P>0.05$). Following phase 2, performance on LC_{TT} was increased in IMT_F only (13.59 ± 2.33 min, absolute increase 0.58 ± 0.65 min, 4.2 ± 4.4%, $P<0.05$, effect size: d=0.24) and was similar post- IMT_{CON} (14.86 ± 2.83 min, $P>0.05$, Figure 6.10). Average speed during LC_{TT} increased in in IMT_F only (10.9 ± 1.9 $km \cdot h^{-1}$, absolute increase: 0.5 $km \cdot h^{-1}$, 4.5%, $P<0.05$).

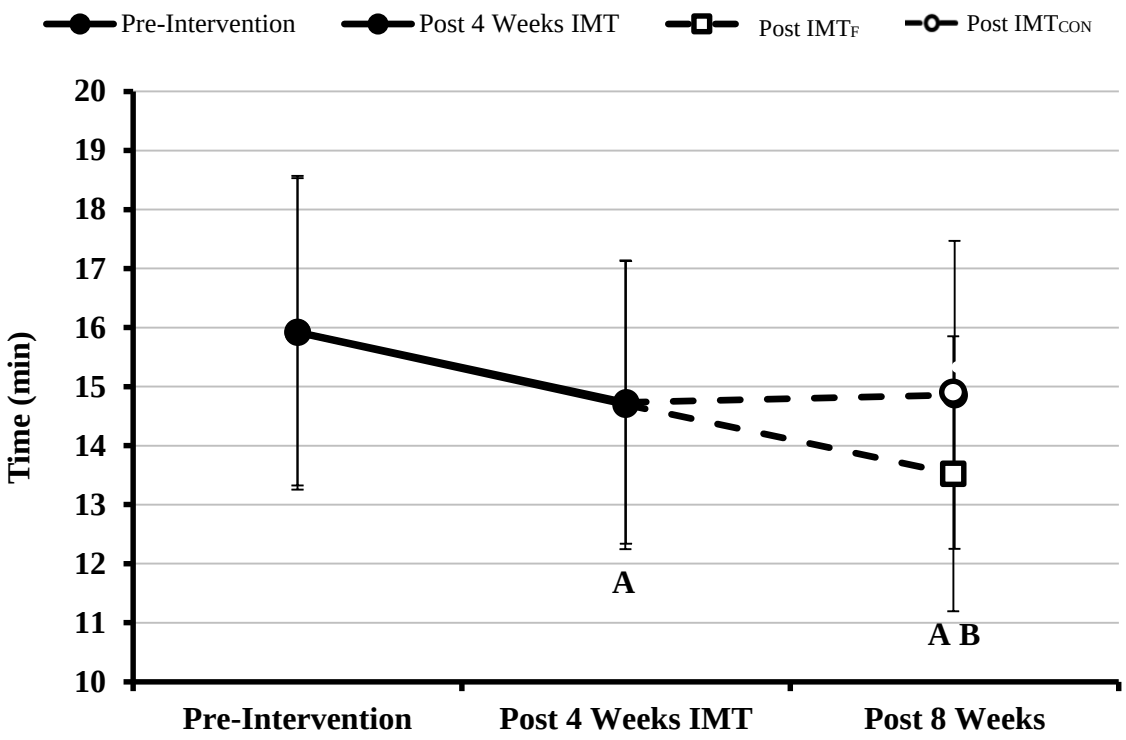

Figure 6.10 Absolute time for LC_{TT} performance, values are presented as mean ± SD. A, different to pre-intervention: B, different to post 4 wks IMT in IMT_F (P<0.05).

6.3.2 SPORT SPECIFIC ENDURANCE PLANK TEST

Mean performance at baseline was 4.11 ± 2.55 min for phase 1 and improved post 4 week IMT 5.06 ± 3.65 min (absolute increase 0.58 ± 1.36 min P<0.05). There was a between group difference once the groups were separated post 4 wk IMT; IMT_F 3.61 ± 1.45 min vs IMT_{CON} 5.73 ± 4.75 min, P<0.05). Following phase 2, post 8 week plank performance increased in IMT_F only (5.13 ± 1.86 min, absolute increase 0.94 ± 1.74 min P<0.05) and was similar in IMT_{CON} (5.44 ± 4.70, P>0.05; see Table 6.2).

6.3.3 RESPIRATORY MUSCLE PRESSURES

Training compliance was high in both groups during phase 1 (94 ± 7%) and phase 2 (IMT_F 94 ± 5% and IMT_{CON} 94 ± 9%) and was similar to the values reported in Chapter 5. Pre-intervention baseline P_{Imax} was 151 ± 36 cmH_2O and reduced to 135 ± 42 cmH_2O post LC (absolute reduction 16 ± 23 cmH_2O, 11% P<0.05), with no further reduction post LC_{TT} (132 ±

38 cmH_2O, P>0.05). In the post-phase 1 trial, relative to pre-intervention baseline values, P_{Imax} was greater at rest (pre 151 ± 36 vs post 172 ± 39 cmH_2O, +14%, P<0.05), post-LC (pre 135 ± 42 vs post 160 ± 36 cmH_2O, +19%, P<0.05) and post-LC_{TT} (pre 132 ± 38 vs post 148 ± 34 cmH_2O, +12%, P<0.05) after IMT. The individual responses to IMT, IMT_F and IMT_{CON} are shown in figure 6.11.There were no between group differences in P_{Imax} after participants were separated into IMT_F or IMT_{CON} for phase two (147 ± 35 cmH_2O and 156 ± 39 cmH_2O, P>0.05 respectively). Following phase 2 during the experimental trial, P_{Imax} was greater at each time point in IMT_F only (P<0.05) and similar in IMT_{CON} (P>0.05) as shown in Figures 6.12 and 6.13 respectively. P_{Imax} increased in IMT_F at baseline 177 ± 25 cmH_2O (absolute increase 11 ± 10 cmH_2O, P<0.05) and post LC 167 ± 28 cmH_2O (absolute increase 8 ± 19 cmH_2O, P<0.05) and was similar to post 4 week values after LC_{TT} (P>0.05, Figure 6.12). P_{Emax} at baseline was similar to post-LC and post LC_{TT} values during phase one and was not different between groups during phase two (Tables 6.3 and 6.4, P>0.05).

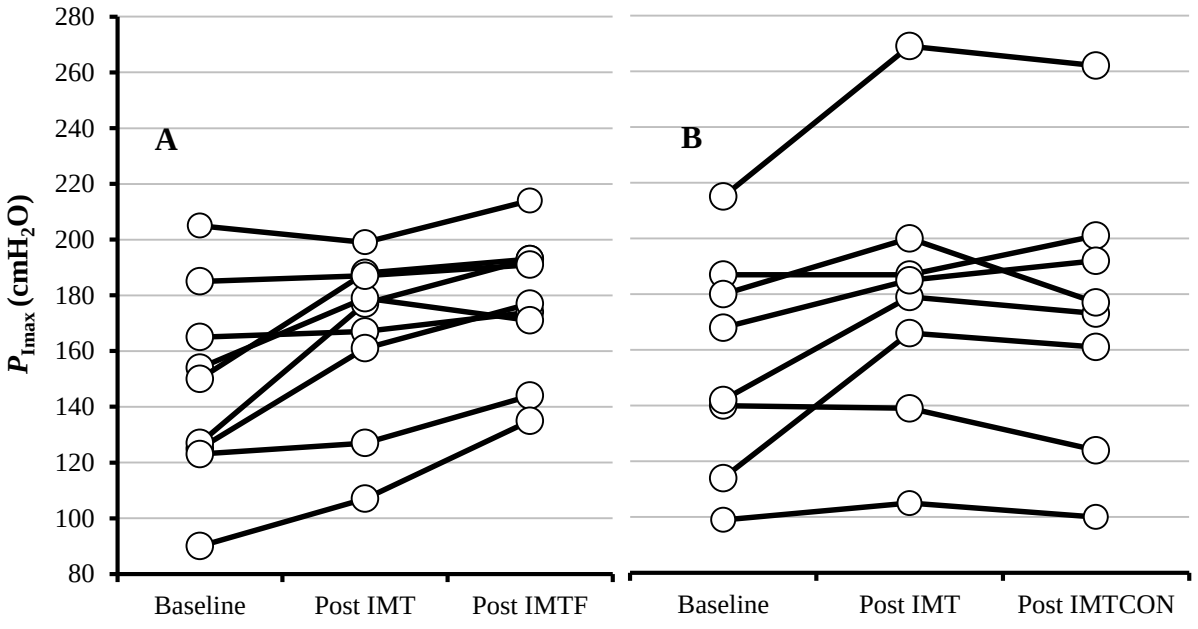

Figure 6.11 Individual changes in maximal inspiratory pressure (P_{Imax}) from baseline, post IMT and post IMT_F (panel A) and post IMT_{CON} (panel B).

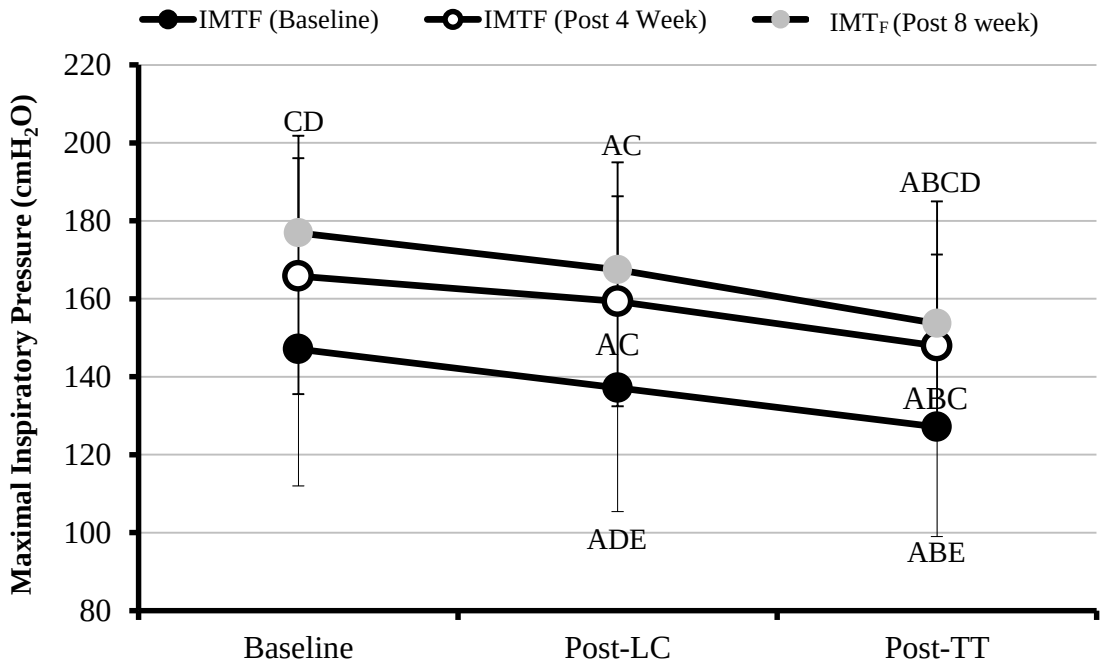

Figure 6.12 Within group and between trial changes of P_{Imax} for IMT_F at baseline, post-LC and post LC_{TT} with values are presented as mean ± SD. A, different to baseline: B, different to post-LC, C, different to pre intervention, D, different to post 4 wks, E, different to post 8 wks.

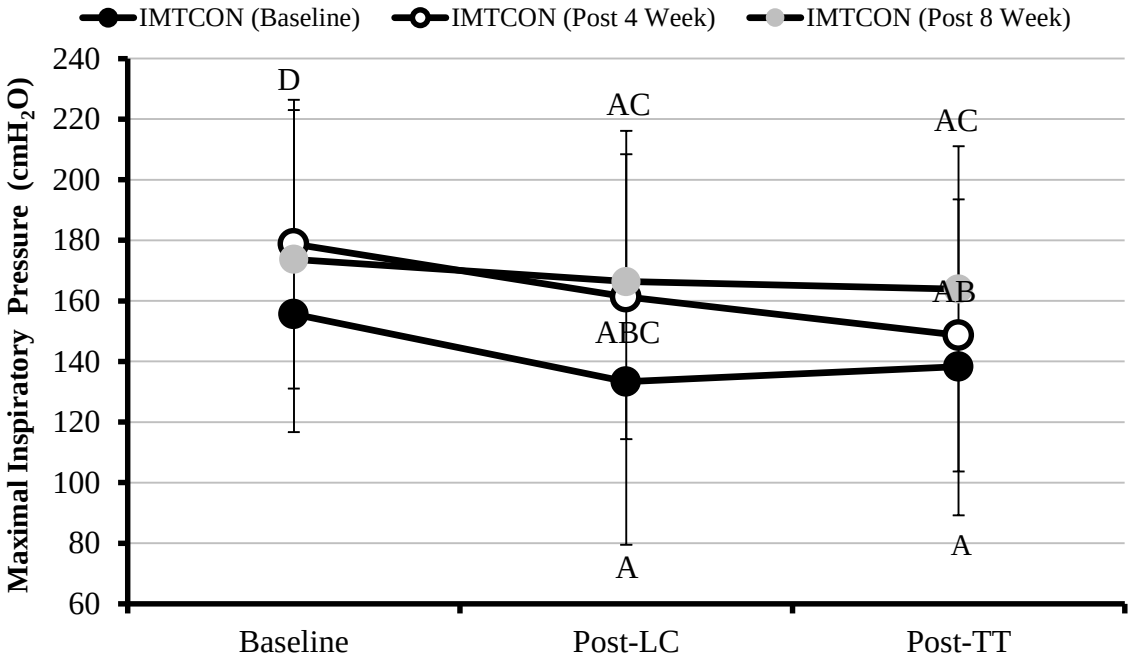

Figure 6.13 Within group changes of P_{Imax} for IMT_{CON}, at baseline, post-LC and post LC_{TT} with values are presented as mean ± SD. A, different to baseline: B, different to post-LC, C, different to pre intervention, D, different to post 4 wks, E, different to post 8 wks.

6.3.4 PULMONARY FUNCTION

Pulmonary function for all participants was not different between groups prior to or post phase 1 or phase 2 interventions (P>0.05). There were also no within experimental trial changes throughout (P>0.05, see Tables 6.2, 6.3 and 6.4).

6.3.5 PHYSIOLOGICAL AND PERCEPTUAL RESPONSES

Physiological and perceptual responses for IMT_F and IMT_{CON} for each trial are shown in Tables 6.3 and 6.4 respectively. In summary, HR increased from rest, post LC and post-LC_{TT} during pre-intervention and post phase 1 of the intervention (P< 0.05). For post phase 2 measures, increases were similar to pre-intervention and post phase 1 IMT and there were no between group differences at any time point throughout the intervention (P>0.05). Breathing pattern ($\dot{V}_T$, f_B and $\dot{V}_E$) increased from rest at each time point during all experimental trials in phase 1 and phase 2 and were unaffected by any intervention. $[Lac^-]_B$ concentrations were similar at rest in each trial (P>0.05) and increased post-LC and post LC_{TT} after each (P<0.05) trial but was unaffected by any intervention (P>0.05). A similar pattern of change was observed for $[glucose^-]_B$ (P>0.05). Perceptual responses were similar at baseline in all trials (P>0.05) and increased post LC and Post LC_{TT} during all trials (P<0.05). There were no within or between group changes in perceptual responses at any time point.

Table 6.2 Performance and physiological data at rest and during LC and LC_{TT}, prior to the intervention and post 4 weeks IMT (phase 1).

	Pre-Intervention			Post 4 WK IMT		
	Baseline	**PLC**	**PLC_{TT}**	**Baseline**	**PLC**	**PLC_{TT}**
Time-trial (min)	-	-	15.93 ± 2.30	-	-	14.73 ± 2.40^{C}
Core Strength (min)	4.11 ± 2.55	-	-	5.06 ± 3.65^{C}	-	-
P_{Imax}	151 ± 36	135 ± 42^{A}	132 ± 38	172 ± 39^{C}	160 ± 36^{AC}	148 ± 34^{ABC}
P_{Emax}	134 ± 54	133 ± 55	131 ± 59	143 ± 60	138 ± 56	133 ± 53
FEV_1 (L)	3.86 ± 0.86	3.92 ± 0.84	3.86 ± 0.84	3.88 ± 0.90	3.92 ± 0.87	3.87 ± 0.91
FVC (L)	4.58 ± 0.84	4.50 ± 0.91	4.40 ± 0.72	4.57 ± 0.68	4.55 ± 0.81	4.40 ± 0.85
FEV_1/ FVC (%)	83 ±11	87 ± 8	89 ± 7	87 ± 8	86 ± 8	90 ± 9
PEF ($L \cdot min^{-1}$)	529 ± 128	525 ± 128	508 ± 138	552 ± 146^{C}	506 ± 147	521 ± 140
HR *(beats·min^{-1})*	78 ± 11	138 ± 19^{A}	182 ± 14^{AB}	85 ± 13	143 ± 21^{A}	183 ± 23^{AB}
$[Lac^-]_B$ ($mmol \cdot l^{-1}$)	1.45 ± 0.58	1.65 ± 0.71	7.45 ± 2.69^{A}	1.61 ± 0.58	1.45 ± 0.80	7.08 ± 2.70^{A}
$[glucose^-]_B$ ($mmol \cdot l^{-1}$)	4.90 ± 1.00	4.58 ± 0.31	5.08 ± 1.14	4.96 ± 1.21	4.51± 0.44	5.14 ± 1.23
$\dot{V}_E$ *(L·min^{-1})*	20.45 ± 6.65	49.11 ± 12.21^{A}	100.41 ± 25.11^{AB}	18.79 ± 7.50	49.42 ± 12.89^{A}	107.19 ± 17.21^{AB}
V_T (L)	0.78 ± 0.19	1.16 ± 0.25^{A}	1.63 ± 0.36^{AB}	0.76 ± 0.14	1.21 ± 0.26^{A}	1.73 ± 0.29^{AB}
f_B ($breaths \cdot min^{-1}$)	26 ± 4	43 ± 8^{A}	61 ± 10^{AB}	26 ± 5	42 ± 9^{A}	63 ± 10^{AB}
$\dot{V}O_2$ *(L·min^{-1})*	0.61 ± 0.25	1.76 ± 0.41^{A}	2.84 ± 0.74^{AB}	0.60 ± 0.11	1.69 ± 0.36^{A}	3.06 ± 0.44^{ABC}
$\dot{V}CO_2$ *(L·min^{-1})*	0.61 ± 0.23	1.83 ± 0.47 ^{A}	3.32 ± 0.64 ^{AB}	0.57 ± 0.16	1.67 ± 0.42 ^{A}	3.45 ± 0.49 ^{AB}
RER	0.98 ± 0.14	0.98 ± 0.06	1.14 ± 0.13 ^{AB}	0.94 ± 0.12	1.00 ± 0.08 ^{A}	1.15 ± 0.11 ^{AB}
RPE (AU)	6 ± 0	12 ± 3 ^{A}	17 ± 2 ^{AB}	6 ± 0	11 ± 3 ^{A}	18 ± 2 ^{AB}
RPE_{legs} *(AU)*	0 ± 1	3 ± 2 ^{A}	7 ± 2 ^{AB}	0 ± 1	3 ± 2 ^{A}	6 ± 2 ^{AB}
$RPE_{breathing}$*(AU)*	0 ± 0	3 ± 2 ^{A}	7 ± 2 ^{AB}	0 ± 0	3 ± 2 ^{A}	7 ± 2 ^{AB}

Maximum inspiratory pressure (P_{Imax}), Maximum expiratory pressure (P_{Emax}), forced vital capacity (FVC), forced expiratory volume in one second (FEV_1), forced expiratory volume in one second / forced vital capacity ratio (FEV1/ FVC), peak expiratory flow (PEF): Heart rate (HR), Blood Lactate [Lac-]B, minute ventilation ($\dot{V}_E$), oxygen consumption ($\dot{V}O_2$), carbon dioxide production ($\dot{V}CO2$), Respiratory exchange ratio (RER), Arbitrary units (AU). A=Different from rest, B= Different from post-LC, C = Different between session.

Table 6.3 Performance and physiological data at rest and during LC and LC_{TT}, post 4 weeks IMT and post IMT_F (phase 2).

	Post 4 WK IMT_F			**Post IMT_F**		
	Baseline	**PLC**	**PLC_{TT}**	**Baseline**	**PLC**	**PLC_{TT}**
Time-trial (min)	-	-	14.11 ± 2.14^{C}	-	-	13.59 ± 2.33^{C}
Core Strength (min)	3.61 ± 1.45	-	-	5.13 ± 1.86^{C}	-	-
P_{Imax}	166 ± 30^{C}	159 ± 27^{AC}	148 ± 23^{ABC}	177 ± 25^{C}	167 ± 28^{AC}	154 ± 31^{ABC}
P_{Emax}	125 ± 27	119 ± 24	116 ± 17	131 ± 28	130 ± 25	119 ± 17
FEV_1 (L)	3.93 ± 0.34	3.98 ± 0.36	3.92 ± 0.30	3.82 ± 0.46	3.99 ± 0.35	3.80 ± 0.28
FVC (L)	4.46 ± 0.28	4.48 ± 0.40	4.30 ± 0.39	4.44 ± 0.32	4.45 ± 0.31	4.21 ± 0.49
FEV_1/ FVC (%)	88 ± 9	89 ± 9	91 ± 10	88 ± 10	89 ± 10	90 ± 8
PEF ($L \cdot min^{-1}$)	542 ± 107	516 ± 88	530 ± 91	547 ± 99	532 ± 96	525 ± 85
HR *(beats·min^{-1})*	83 ± 12	143 ± 21^{A}	189 ± 12^{AB}	79 ± 23	137 ± 22^{A}	191 ± 10^{AB}
$[Lac^-]_B$ ($mmol \cdot l^{-1}$)	1.27 ± 0.36	1.49 ± 1.09	7.36 ± 2.66^{A}	1.28 ± 0.44	1.40 ± 0.45	7.33 ± 1.38^{A}
$[glucose^-]_B$ ($mmol \cdot l^{-1}$)	4.60 ± 0.73	4.54 ± 0.52	5.63 ± 1.07	4.60 ± 0.62	4.45 ± 0.32	5.49 ± 1.04
$\dot{V}_E$ *(L·min^{-1})*	16.6 ± 6.8	48.2 ± 9.6^{A}	109.3 ± 16.8^{AB}	19.8 ± 4.0	45.2 ± 11.0^{A}	106.9 ± 24.5^{AB}
V_T (L)	0.8 ± 0.2	1.1 ± 0.3^{A}	1.7 ± 0.3^{AB}	0.7 ± 0.1	1.1 ± 0.2^{A}	1.6 ± 0.4^{AB}
f_B ($breaths \cdot min^{-1}$)	25 ± 2	43 ± 6^{A}	65 ± 12^{AB}	30 ± 8	43 ± 6^{A}	66 ± 9^{AB}
$\dot{V}O_2$ *(L·min^{-1})*	0.58 ± 0.10	1.64 ± 0.20^{A}	3.00 ± 0.29^{AB}	0.62 ± 0.11	1.60 ± 0.25^{A}	2.71 ± 0.86^{AB}
$\dot{V}CO_2$ *(L·min^{-1})*	0.55 ± 0.14	1.60 ± 0.35^{A}	3.48 ± 0.42^{AB}	0.58 ± 0.12	1.54 ± 0.32^{A}	3.42 ± 0.84^{AB}
RER	0.93 ± 0.10	1.01 ± 0.09	1.16 ± 0.09^{AB}	0.93 ± 0.08	0.96 ± 0.09	1.16 ± 0.14^{AB}
RPE (AU)	6 ± 0	11 ± 3^{A}	18 ± 2^{AB}	6 ± 0	11 ± 3^{A}	18 ± 2^{AB}
RPE_{legs} *(AU)*	0 ± 1	3 ± 2^{A}	8 ± 2^{AB}	0 ± 0	2 ± 1^{AC}	8 ± 1^{AB}
$RPE_{breathing}$*(AU)*	0 ± 0	3 ± 1^{A}	8 ± 2^{AB}	0 ± 0	2 ± 1^{AC}	8 ± 2^{AB}

Maximum inspiratory pressure (P_{Imax}), Maximum expiratory pressure (P_{Emax}), forced vital capacity (FVC), forced expiratory volume in one second (FEV_1), forced expiratory volume in one second / forced vital capacity ratio (FEV1/ FVC), peak expiratory flow (PEF): Heart rate (HR), Blood Lactate [Lac-]B, minute ventilation ($\dot{V}_E$), oxygen consumption ($\dot{V}O_2$), carbon dioxide production ($\dot{V}CO2$), Respiratory exchange ratio (RER), Arbitrary units (AU). A=Different from rest, B= Different from post-LC, C = Different between session.

Table 6.4 Performance and physiological data for IMT_{CON} group during LC and LC_{TT}, prior to and post IMT_{CON} (phase 2).

	Post 4 WK IMT_{CON}			Post IMT_{CON}		
	Baseline	**PLC**	**PLC_{TT}**	**Baseline**	**PLC**	**PLC_{TT}**
Time-trial (min)	-	-	15.44 ± 2.61^{C}	-	-	14.86 ± 2.83^{C}
Core Strength (min)	5.73 ± 4.75	-	-	5.44 ± 5.24	-	-
P_{Imax}	179 ± 48^{C}	161 ± 47^{AC}	149 ± 45^{AB}	174 ± 49^{C}	166 ± 50^{A}	164 ± 47^{C}
P_{Emax}	163 ± 81	160 ± 74	152 ± 73	168 ± 80	158 ± 70	158 ± 69
FEV_1 (L)	3.82 ± 1.30	3.85 ± 1.26	3.82 ± 1.34	3.90 ± 0.91	3.87 ± 0.97	3.89 ± 1.05
FVC (L)	4.69 ± 0.97	4.63 ± 1.15	4.50 ± 1.20	4.54 ± 1.18	4.46 ± 1.21	4.36 ± 1.18
FEV_1/ FVC (%)	85 ± 6	86 ± 7	88 ± 8	86 ± 8	87 ± 7	88 ± 7
PEF ($L \cdot min^{-1}$)	580 ± 91	564 ± 115	564 ± 119	563 ± 104	550 ± 122	567 ± 106
HR *(beats·min^{-1})*	88 ± 15	143 ± 23^{A}	176 ± 31^{AB}	88 ± 10	139 ± 15^{A}	186 ± 11^{AB}
$[Lac^-]_B$ ($mmol \cdot l^{-1}$)	1.99 ± 0.56	1.40 ± 0.34	6.78 ± 2.90^{AB}	1.28 ± 0.44	2.03 ± 1.07^{A}	6.78 ± 1.88^{AB}
$[glucose^-]_B$ ($mmol \cdot l^{-1}$)	5.38 ± 1.54	4.49 ± 0.38	4.58 ± 1.22	4.61 ± 0.39	3.34 ± 0.47	5.09 ± 1.46^{B}
$\dot{V}_E$ *(L·min^{-1})*	21.3 ± 7.9	50.8 ± 16.4^{A}	104.8 ± 18.5^{AB}	26.4 ± 5.7	50.2 ± 13.1^{A}	107.2 ± 15.4^{AB}
V_T (L)	0.8 ± 0.1	1.3 ± 0.2^{A}	1.8 ± 0.3	1.0 ± 0.3	1.3 ± 0.3^{A}	1.8 ± 0.3^{AB}
f_B ($breaths \cdot min^{-1}$)	28 ± 7	39 ± 11^{A}	60 ± 6^{AB}	29 ± 5	40 ± 10^{A}	59 ± 7^{AB}
$\dot{V}O_2$ *(L·min^{-1})*	0.63 ± 0.12	1.76 ± 0.49^{A}	3.13 ± 0.57^{AB}	0.85 ± 0.22	1.79 ± 0.38^{A}	3.17 ± 0.50^{AB}
$\dot{V}CO_2$ *(L·min^{-1})*	0.60 ± 0.18	1.75 ± 0.51^{A}	3.41 ± 0.58^{AB}	0.77 ± 0.18	1.74 ± 0.44^{A}	3.55 ± 0.59^{AB}
RER	0.94 ± 0.13	1.00 ± 0.07	1.13 ± 0.13^{AB}	0.92 ± 0.06	0.97 ± 0.06	1.12 ± 0.06^{AB}
RPE (AU)	6 ± 0	$11 \ 4^{A}$	17 ± 2^{AB}	6 ± 0	10 ± 4^{A}	17 ± 2^{AB}
RPE_{legs} (AU)	0 ± 1	2 ± 2^{A}	5 ± 2^{AB}	0 ± 1	2 ± 2^{A}	6 ± 2^{AB}
$RPE_{breathing}$(AU)	0 ± 0	2 ± 2^{A}	6 ± 1^{AB}	0 ± 0	3 ± 2^{A}	7 ± 2^{AB}

Maximum inspiratory pressure (P_{Imax}), Maximum expiratory pressure (P_{Emax}), forced vital capacity (FVC), forced expiratory volume in one second (FEV_1), forced expiratory volume in one second / forced vital capacity ratio (FEV1/ FVC), peak expiratory flow (PEF): Heart rate (HR), Blood Lactate [Lac-]B, minute ventilation ($\dot{V}_E$), oxygen consumption ($\dot{V}O_2$), carbon dioxide production ($\dot{V}CO2$), Respiratory exchange ratio (RER), Arbitrary units (AU). A=Different from rest, B= Different from post-LC, C = Different between session.

6.3.6 VOLITIONAL AND EVOKED FORCE

6.3.6.1 MAXIMUM VOLUNTARY CONTRACTION

MVC peak force was similar at rest during all trials (Tables 6.5, 6.6 and 6.7, $P>0.05$). During phase 1 prior to the intervention, MVC peak force was reduced from rest (561 ± 116 N) post LC (534 ± 105 N, absolute reduction 42 ± 39 N, 7%, $P<0.05$), post LC_{TT} (534 ± 114 N, absolute reduction 28 ± 90 N, 7%, $P<0.05$) and again $post_{30min}$ min (494 ± 119 N, absolute reduction 68 ± 74 N, 11%, $P<0.05$). There was no change post LC vs post LC_{TT} or post LC_{TT} vs $post_{30min}$ ($P>0.05$). Post 4 week IMT, MVC peak force reduced similarly to pre intervention values from baseline (554 ± 127 N), post LC (514 ± 109 N, absolute reduction 40 ± 75 N, 6%, $P<0.05$), post LC_{TT} (511 ± 122 N, absolute reduction 44 ± 84 N, 6%, $P<0.05$) and again $post_{30min}$ (483 ± 126 N, absolute reduction 72 ± 97 N, 12%, $P<0.05$).

During phase 2, there was no between group differences in MVC peak force at rest, ($P>0.05$). Relative to phase one baseline values in IMT_F (566 ± 56 N), peak force was reduced post LC (516 ± 85 N, absolute reduction 50 ± 51 N, 9 ± 9%, $P>0.05$); however, force was similar post LC_{TT} (518 ± 69 N, absolute reduction 49 ± 47 N, 9 ± 8%, $P<0.05$) and $post_{30min}$ (501 ± 73 N, absolute reduction 66 ± 49 N, 12 ± 8%, $P<0.05$). There was no change in IMT_{CON} across any time point (See Table 6.7, $P<0.05$) and there were also no between group differences at any time point when comparing phase one values to post IMT_F and IMT_{CON} ($P>0.05$).

Voluntary activation (%VA) is displayed in Tables 6.5, 6.6 and 6.7. During phase one %VA was reduced from baseline (92 ± 11%) at each time point ($P<0.05$) with reductions post LC and post LC_{TT} values (absolute reduction 14 ± 27%, $P<0.05$); however, there were no changes when comparing post LC_{TT} to $post_{30min}$ ($P>0.05$). During phase two reductions were

similar to phase 1 and additionally there were no between group differences when the groups were split for phase 2, or during the post 8 week trial (P>0.05).

6.3.6.2 TWITCH FORCE

Peak twitch force was similar at baseline prior to and following each phase of the intervention (Tables 6.5, 6.6 and 6.7, P>0.05). Relative to baseline values peak twitch force was similar post LC (P>0.05) and reduced post LC_{TT} (132 ± 34 N, absolute reduction 18 ± 24 N, 10%, P<0.05). Peak twitch force increased $post_{30min}$ relative to LC_{TT} values (148 ± 29 N, absolute increase 17 ± 15 N, 14%, P<0.05), and returned to rest (P>0.05). Post phase 1, peak twitch force was similar post LC when compared with resting values (Table 6.5) and was reduced post LC_{TT} (123 ± 27 N, absolute reduction 14 ± 28 N, 9%, P<0.05). Post phase 2, there were no within or between session differences in IMT_F or $IMT_{CON,}$ see Tables 6.6 and 6.7.

6.3.6.3 INTERPOLATED DOUBLET

Peak force values for baseline, IMT_F and IMT_{CON} for the potentiated doublet are shown in Tables 6.6 and 6.7 respectively. In summary, there were no changes within experimental trials during phase 1 or phase 2 and there were no differences in either IMT_F or IMT_{CON} during phase 2 of the intervention (P>0.05).

6.3.6.4 20HZ AND 50HZ STIMULATION

Peak force for both 20 Hz and 50 Hz stimulations were not different during baseline or any subsequent experimental trial (P>0.05). Within session changes for both IMT_F and IMT_{CON} are shown in Tables 6.6 and 6.7 respectively; in summary, there were no within or between session changes in either group (P>0.05).

6.3.6.5 20:50 HZ RATIO

20 Hz and 50 Hz ratio was similar between groups during phase two trials (Tables 6.6 and 6.7, $P>0.05$). Prior to the intervention and relative to baseline (0.81 ± 0.08) 20 Hz: 50 Hz ratio was unchanged post LC (0.79 ± 0.07, $P>0.05$). The ratio was however reduced post LC_{TT} (0.72 ± 0.08, absolute reduction 0.09 ± 0.05, $P<0.05$) and $post_{30min}$ (0.74 ± 0.09, absolute reduction 0.11 ± 0.19, $P<0.05$). Post 4 weeks IMT, the ratio was unchanged at all time-points (Table 6.5), and there were no within or between group changes post 8 weeks.

Table 6.5 Isometric and electrically evoked stimulation parameters at baseline, post LC, post LC_{TT} and $post_{30min}$ during pre-intervention and post 4 week IMT trials, data here is pooled for both groups.

	Pre-Intervention				Post 4 WK IMT			
	Baseline	**Post LC**	**Post LC_{TT}**	**$Post_{30min}$**	**Baseline**	**Post LC**	**Post LC_{TT}**	**$Post_{30min}$**
Twitch Peak Force (N)	149 ± 33	148 ± 34	132 ± 28	148 ± 29	130 ± 35	138 ± 37	123 ± 27	135 ± 38
VA (%)	92 ± 11	79 ± 21	66 ± 18	72 ± 19	90 ± 8	78 ± 71	76 ± 15	76 ± 14
MVC Peak Force (N)	561 ± 116	534 ± 105	534 ± 114	494 ± 119	554 ± 127	514 ± 109	511 ± 122	483 ± 126
Doublet Peak Force (N)	349 ± 96	358 ± 102	329 ± 86	342 ± 82	305 ± 47	324 ± 53	314 ± 61	309 ± 65
20 Hz Peak Force (N)	362 ± 98	379 ± 81	327 ± 74	351 ± 92	337 ± 97	346 ± 86	309 ± 81	309 ± 82
50 Hz Peak Force (N)	447 ± 109	482 ± 100	454 ± 96	452 ± 87	417 ± 107	445 ± 97	421 ± 100	416 ± 91
20:50 Ratio	81 ± 8	79 ± 7	72 ± 8	74 ± 9	81 ± 6	78 ± 7	73 ± 8	74 ± 9

Values are presented as mean ± SD. Voluntary activation, VA, Maximum voluntary contraction MVC.

Table 6.6 Isometric and electrically evoked stimulation parameters at baseline, post LC, post LC_{TT} and $post_{30min}$ during post 4 week IMT trials (IMT_F group data only) and post IMT_F trial.

	Post 4 WK IMT_F				Post IMT_F			
	Baseline	**PLC**	**PLC_{TT}**	**$Post_{30min}$**	**Baseline**	**PLC**	**PLC_{TT}**	**$Post_{30min}$**
Twitch Peak Force (N)	127 ± 31	135 ± 28	120 ± 20	137 ± 31	133 ± 33	133 ± 27	113 ± 25	131 ± 32
VA (%)	90 ± 3	82 ± 5	73 ± 7	74 ± 12	90 ± 12	86 ± 8	77 ± 14	82 ± 12
MVC Peak Force (N)	560 ± 87	499 ± 87	482 ± 105	454 ± 116	566 ± 56	516 ± 85	518 ± 69	501 ± 73
Doublet Peak Force (N)	307 ± 51	331 ± 58	319 ± 68	313 ± 68	313 ± 54	314 ± 55	294 ± 49	287 ± 42
20 Hz Peak Force (N)	331 ± 73	345 ± 64	303 ± 50	303 ± 61	340 ± 73	341 ± 81	304 ± 73	306 ± 64
50 Hz Peak Force (N)	407 ± 87	438 ± 84	410 ± 79	400 ± 76	408 ± 79	404 ± 85	404 ± 80	401 ± 65
20:50 Ratio	0.81 ± 0.04	0.78 ± 0.07	0.74 ± 0.08	0.74 ± 0.08	0.80 ± 0.08	0.82 ± 0..06	0.75 ± 0.09	0.76 ± 0.09

Values are presented as mean ± SD. Voluntary activation, VA, Maximum voluntary contraction MVC.

Table 6.7 Isometric and electrically evoked stimulation parameters at baseline, post LC, post LC_{TT} and $post_{30min}$ during post 4 week IMT trials (IMT_{CON} group data only) and post IMT_{CON} trial.

	POST 4 WK IMT_{CON}				Post IMT_{CON}			
	Baseline	**PLC**	**PLC_{TT}**	**$Post_{30min}$**	**Baseline**	**PLC**	**PLC_{TT}**	**$Post_{30min}$**
Twitch Peak Force (N)	133 ± 41	141 ± 47	127 ± 33	133 ± 47	141 ± 50	145 ± 44	128 ± 43	143 ± 43
VA (%)	90 ± 11	74 ± 26	70 ± 22	80 ± 17	89 ± 27	90 ± 18	81 ± 7	76 ± 19
MVC Peak Force (N)	548 ± 167	531 ± 133	542 ± 138	515 ± 136	590 ± 131	566 ± 147	592 ± 143	531 ± 145
Doublet Peak Force (N)	303 ± 45	316 ± 50	309 ± 56	304 ± 65	310 ± 54	330 ± 39	298 ± 58	306 ± 61
20 Hz Peak Force (N)	344 ± 123	348 ± 110	315 ± 110	316 ± 105	351 ± 135	364 ± 108	320 ± 102	328 ± 91
50 Hz Peak Force (N)	427 ± 131	454 ± 116	434 ± 125	435 ± 108	426 ± 148	470 ± 136	426 ± 125	434 ± 117
20:50 Ratio	0.80 ± 0.07	0.76 ± 0.07	0.72 ± 0.08	0.71 ± 0.09	0.81 ± 0.05	0.77 ± 0.06	0.74 ± 0.04	0.79 ± 0.08

Values are presented as mean ± SD. Voluntary activation, VA, Maximum voluntary contraction MVC.

6.4 DISCUSSION

The key findings of this study were fourfold: 1) Phase one: Four weeks IMT improved LC_{TT} performance confirming the findings of Chapter 5, 2) Phase two: IMT_F further improved LC_{TT} performance and IMT_{CON} preserved LC_{TT} performance, 3) Load carriage did not result in fatigue of the m.quadriceps, and finally 4) Neither IMT_F or IMT_{CON} attenuated respiratory muscle fatigue.

6.4.1 TIME-TRIAL PERFORMANCE

LC_{TT} was similar to Chapters 3 and 4. Time-trial performance was significantly improved post 4 weeks IMT (pooled increase 7.5%), which is similar to the improvements observed in Chapter 5. To date, these two studies contain the only other findings to compare the IMT mediated improvement in load carriage performance and further demonstrate that IMT has a genuine ergogenic effect upon running performance carrying a 25 kg thoracic load. This study also demonstrates for the first time that performance can be enhanced further using IMT_F with a further 4% improvement in time-trial performance ($P<0.05$). To date, there has been one similarly designed study to adopt IMT_F which also observed improvements in endurance running performance (3%) beyond an initial 21% improvement after 4 wk IMT (Tong, McConnell, et al., 2014). In this latter study, participants were recreational runners and completed a 4 wk period of foundation IMT before being split into either IMT_F or a control group, with both groups also completing whole-body interval training. Following 6 wks of concurrent interval training and core muscle training, performance on a 60 min treadmill test was improved in both groups, but to a greater extent in the core muscle training group (3.1%) compared with the control group (1.5%). Core muscle endurance per se was only improved in the functional training group (Tong, McConnell, et al., 2014). The additional improvement in performance observed within the

functional training group was attributed to an increase respiratory muscle strength, which has a role in contributing to core muscle strength with these muscles functionally relevant to running performance (Tong, McConnell, et al., 2014). It is possible therefore that the greater improvement in performance observed in the present study was also due to a greater activation/adaptation of respiratory muscles, which also play a key role in stabilising the core, preserving/facilitating the respiratory muscle contribution to ventilation. In addition, this is the first known study to demonstrate that the ergogenic effect of IMT is maintained following a period of maintenance IMT where the training frequency is considerably reduced by 57%. Previously, it was demonstrated that inspiratory muscle function is preserved above baseline following 9 and 18 wk maintenance IMT, but there was no measure of performance (Romer & McConnell, 2003). Thus, this study demonstrates for the first time that the ergogenic effect of IMT can be maintained after initial conditioning, whether this would be maintained after a longer phase of maintenance remains to be determined especially as changes in P_{Imax} and performance are not correlated (Johnson et al., 2007).

6.4.2 RESPIRATORY MUSCLE FUNCTION

P_{Imax} increased 14% following phase one IMT which is lower than previously observed increases in Chapter 5 and in previous IMT studies (HajGhanbari et al., 2013; Illi et al., 2012) and could be a result of the reduced 4 wk training period compared to 6 weeks in Chapter 5 and in previous studies (HajGhanbari et al., 2013; Illi et al., 2012). This could also be the result of increased baseline levels of P_{Imax} when compared with Chapter 5 ($P<0.05$), which as discussed by Brown et al. (2014) could limit possible improvements obtained via IMT methods as the window for physiological adaptation is reduced. The four week period was intended to replicate the study of Tong et al. (2014) who observed a 21% increase in inspiratory muscle strength post 4 week IMT and was adopted to control for the established

ergogenic effects of IMT in both IMT_F and IMT_{CON} and to also prepare a strong foundation within the inspiratory musculature during subsequent IMT_F sessions (Tong, McConnell, et al., 2014). Following IMT_F there was a further, albeit non-significant ($P > 0.05$, $d = 0.40$) increase in P_{Imax} (7%) with IMT_{CON} unchanged. It should be noted that there was a fourfold difference in training volume during IMT_F (1548 breathes) and IMT_{CON} (360 breathes) during phase 2. The little additional improvement in P_{Imax} is in line with the findings of Tong et al. (2014) who suggest reduced training volume and a constant inspiratory load may be responsible for the plateau in $P_{Imax.}$ Training intensity here was adjusted bi-weekly to maintain relative training stimuli throughout the intervention and could explain the minimal but observable difference in P_{Imax} observed post IMT_F (Figure 6.12).

Similar to the findings in Chapter 5, respiratory muscle fatigue was not attenuated following either phase of the intervention, which is demonstrated by the unchanged relative reduction post exercise despite increased absolute strength at each time point ($P < 0.05$). This again contradicts findings within existing literature that have observed attenuated respiratory muscle fatigue following a period of IMT (for a review see HajGhanbari et al., 2013; Illi et al., 2012). It also further rejects the suggestion in Section 5.4.3 that IMT failed to attenuate inspiratory muscle fatigue associated with non-respiratory roles such as supporting posture and spinal stability as core stability and performance on SEPT was increased (Table 6.3 and 6.4). An explanation could be that IMT techniques were initiated from residual volume and participants seek to maximise V_T during all efforts (McConnell, 2011). These were completed without thoracic restriction which inhibits operational lung volumes and as described throughout this thesis reduces EILV and alters the length tension relationship of the respiratory musculature (Brown & McConnell, 2012). Although combining IMT with core muscle training did seek to reduce operational lung volumes, the exercises used sought primarily to reduce EELV and not EILV, which is constrained by the presence of a load.

Consequently, the training stimuli may be targeting and strengthening the inspiratory muscles throughout an operational range, which may not be unitised during exercise with load carriage (seen via increased P_{Imax}, Figure 6.12). It does not however, adequately account for the shift in the boundaries of their length-tension curve (Romer & McConnell, 2004) and is not sufficient to deter RMF. Future research should seek to conduct both IMT and IMT_F under conditions similar to that imposed by the presence of the load essentially by wearing a heavy backpack throughout the training. Alternatively, it may not be IMT, IMT_F or core training that provide a training stimulus to the synergistic respiratory muscle of the thorax which are activated when carrying a load. Concurrent supplementary resistance training of the muscles of the thorax might therefore be required, since muscle fatigue is determined by both sustained rhythmic contractions and the force exerted, aerobic fitness plays no role in this (Bundle & Weyand, 2012). Respiratory muscle fatigue was unchanged at all-time points throughout the intervention, the total work performed and therefore force exerted by the accessory inspiratory muscles may have regulated the global force exerted during Mueller manoeuvres. It was recently reported that the force generated during a Mueller manoeuvre both before and after IMT is regulated by the weakest inspiratory muscle (the chest wall muscles) and not the diaphragm (Brown et al., 2014). Therefore, it is possible that fatigue of the synergistic extra-thoracic muscles resulted in a greater requirement of the chest wall muscle to produce force, which resulted in their greater fatigue thereby limiting greater force production of the diaphragm at all-time points. Accordingly, whether fatigue of this muscle group (the diaphragm and/or chest wall muscles) was truly reduced remains to be determined and should be investigated in the future using non volitional muscle stimulation techniques.

Respiratory muscle fatigue is associated with reduced postural control during unloaded exercise (Janssens et al., 2010) and is exacerbated with thoracic loads due to increased postural sway; therefore, increasing diaphragm activation (Hodges et al., 2001).

Although this has yet to be quantified in a load carriage setting, it is understood that bearing external loads places excessive demands upon the trunk and challenges ventilatory mechanics, leading to both diaphragm and expiratory muscle fatigue as observed in Chapters 3, 4 and 5. This suggests that IMT increases both P_{Imax} and core strength, which is further increased with $IMT_{F,}$ and challenges both inspiration during tasks and core stabilisation, and as witnessed here and in one published study leads to increased strength of both muscles (Tong, McConnell, et al., 2014). Subsequently, IMT_F can enhance the synergy and coordination of breathing and stabilisation between both groups and effectively reduce the relative competition during exercise, leading to increased exercise performance in both unloaded (Tong, McConnell, et al., 2014) and, here for the first time in load carriage activities.

6.4.3 CORE MUSCLE FUNCTION

To coincide with increased LC_{TT} performance there was a 23% improvement following phase one ($P<0.05$) and a further improvement of 58% increase in SEPT performance following phase two but in IMT_F only ($P< 0.05$,). The improvements in core muscle strength correlate with increased P_{Imax} post IMT_F ($r = 0.72$, $P<0.05$); however, no relationships were present between increased performance on SEPT and LC_{TT} ($P>0.05$) shown in Figure 6.14. The improvement in LC_{TT} is therefore attributed to increased P_{Imax}, core strength and the co-ordination between core muscle activation and breathing activities which are targeted during IMT_F activities and requires the utilisation of similar muscle groups as they are tasked with thoracic excursion and trunk stabilisation (Hodges et al., 2005; Janssens et al., 2010b; Tong & Fu, 2006). These have been previously identified as a limiting factor to running performance when the core muscles of endurance runners were fatigued prior to treadmill exercise, resulting in a 30.5% and 39.2% reduction in both SEPT and time

running at 85% $\dot{V}O_2$ peak respectively (Tong, Wu, Nie, Baker, & Lin, 2014). This is a result of the diaphragm's dual role in powering ventilation and providing stability of the trunk during exercise tasks due to the diaphragms insertion on the thoracic (T12) and lumbar (L1 and L2) regions of the spine (Hodges et al., 2005). This increased activation during whole body tasks that challenge stability of the torso and spinal regions which is likely to be exacerbated when bearing a load (Heller et al., 2009). A key mechanism illustrated here sees an increase in intra-abdominal pressure that results from increased activation of the diaphragm during exercise tasks that require increased spinal stiffness. This occurs via its anatomical connections with the lumbar spine which subsequently increases postural control (Hodges & Gandevia, 2000). Janssens et al., (2015) demonstrated for the first time that enhanced inspiratory muscle strength from IMT is accompanied by reductions in expiratory/trunk muscle activity improving flexibility, which is known to compromise postural control.

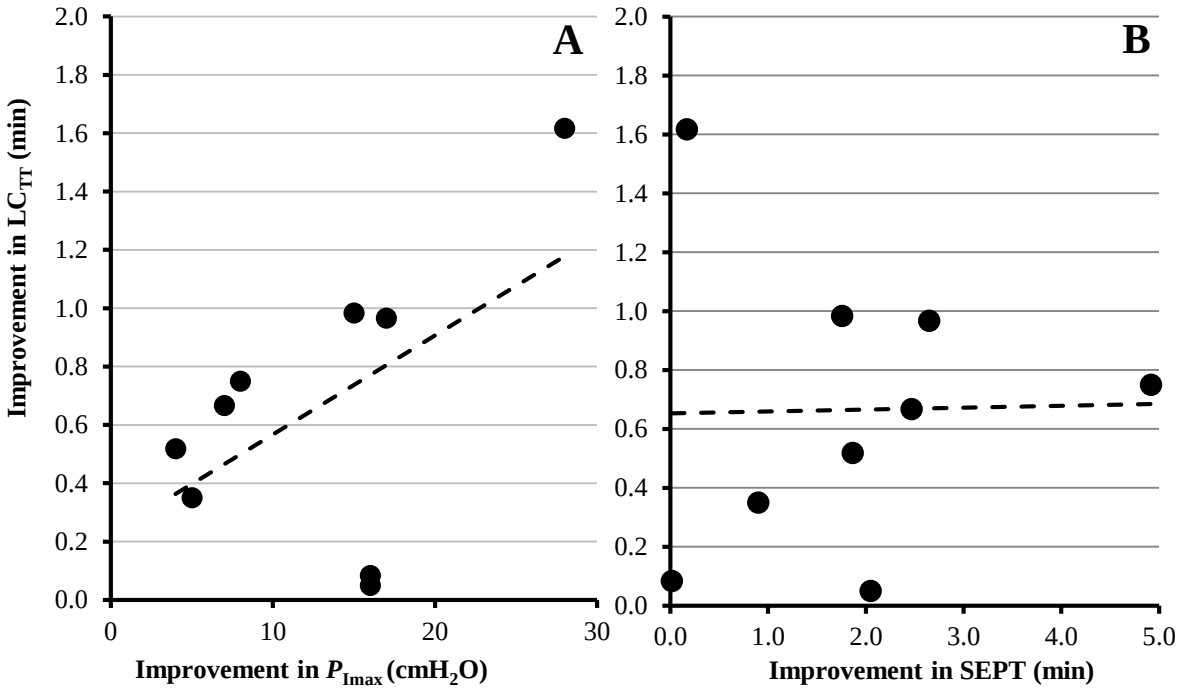

Figure 6.14 A) Correlation for ΔP_{Imax} and ΔLC_{TT} following IMT_F (r=0.528, P<0.05); B) *ΔSEPT* and ΔLC_{TT} following IMT_F (r= 0.019, P>0.05).

6.4.4 PHYSIOLOGICAL MECHANISMS OF IMPROVED IMT_F PERFORMANCE

Comparable increases in P_{Imax} and LC_{TT} performance were observed in IMT following phase one, which again were similar with additional reductions in perceptual responses post-LC and IMT_F only during phase two ($P< 0.05$); however no other reductions in HR or other physiological variables were observed that are typically associated with the use of chronic loading of the inspiratory muscles. This may in part be explained by reduced training duration for the IMT program: the training intervention used in Chapter 5 was six weeks in length compared with four weeks here, which may seek to augment cardiovascular adaptations. Although no cardiovascular adaptations were seen here, the competition for $\dot{Q}$ during whole body exercise manifests in a trade off during exercise, a relationship that favours the respiratory musculature to assist with thoracic excursion during times of peak ventilation (Dempsey, Romer, Rodman, Miller, & Smith, 2006). The suggestion of a respiratory muscle metaboreflex was speculated in Chapter 5 and is apparent from existing research (Sheel et al., 2001; Sheel, Derchak, Pegelow, & Dempsey, 2002), where a sympathetically mediated redistribution of $\dot{Q}$ through limb vasoconstriction. This is unlikely to occur during load carriage activities, despite reductions in the critical threshold for respiratory muscle fatigue observed in Chapter 3 (~59% $\dot{V}O_2$ peak). It was suggested in Chapter 5 that increased O_2 extraction would occur in the peripheries sustaining limb function despite any alteration is limb vascular conductance (Jones et al., 2011; Romer et al., 2006). The data here also supports this notion as there were no reductions in peak force on the isometric voluntary and electrically evoked contractions (Tables 6.5, 6.6 and 6.7). Despite observable reductions in knee extensor force post LC in all trials, this was non-significant ($P>0.05$) and is likely due to large variation within the participant sample. The reductions observed here were lower than those observed within existing literature (Blacker, Fallowfield, Bilzon, et al., 2013) and can be explained by a difference in methodology, as in

this study (2010) participants completed 120 min of LC with 25 kg load in comparison to the 60 min completed within this thesis. It can therefore be suggested that completing LC here was not sufficient in eliciting neuromuscular fatigue at a central or peripheral level. It is anticipated however that sustaining this for prolonged periods of time (~2 hours) would result in altered contractile function and reduced peak force of the m. quadriceps (Blacker, Fallowfield, Bilzon, et al., 2013; Blacker, Fallowfield, et al., 2010). This is caused by increased activation of m. quadriceps due to both postural adaptations; namely increased hip flexion, which increases eccentric loading and shortens the m. quadriceps reducing its optimal length for contraction (Knapik et al., 1996), and secondly, via increased ground reaction forces that imposes further strain on m.quadriceps increasing the total force that must be absorbed by the m. quadriceps (Tilbury-Davis & Hooper, 1999). This increase in contractile activity during prolonged load carriage marching results in peripheral neuromuscular fatigue, muscle damage and more specifically, a reduction in the excitation contraction coupling within the muscle (Blacker, Fallowfield, et al., 2010). However, further research is required to understand the origins and global effects of neuromuscular fatigue.

The only parameter from the non-volitional measures to change was voluntary activation and is derived from the interpolated doublet and was reduced post LC and LC_{TT} in all trials. The reductions post LC are in line with previous load carriage research (Blacker, Fallowfield, Bilzon, et al., 2013), but it is the first instance that this has been assessed following a maximal self-paced time trial. Reduced voluntary activation is indicative of central fatigue and reduced central motor drive (Taylor, 2009), and it is suggested that this reduction seeks to protect the muscle from further intracellular damage by reduced action potential propagation to the neuromuscular junction (Noakes, 2007), thus reducing both motor unit recruitment and limiting peak force. This is overcome with a superimposed

electrical stimulation direct to the m. quadriceps, artificially stimulating motor units and eliciting a greater peak force (Edwards et al., 1977).

The reduction here is surprising as no change was observed in peak force values derived from MVC contractions, and could potentially be explained by a potentiation effect. This can last from 1 to 10 minutes after a maximal contraction, and is characterised by transient increases in the mechanical response to stimulation resulting from previous contractile activity (Sheel & Romer, 2012). Despite the electrical stimulation protocol demonstrating exceptional intra and inter-day reliability as described in Section 6.3.6 and elsewhere (Blacker, Fallowfield, & Willems, 2013), these measures were conducted in the absence of fatiguing exercise and have only been used to quantify the response to prolonged sub-maximal treadmill marching (Blacker, Fallowfield, et al., 2010). This is the first known study to investigate the neuromuscular response to both sub-maximal and maximal load carriage exercise where the potentiation effect is not identified.

The data also presented in Tables 6.5, 6.6 and 6.7 demonstrate large variations in peak MVC force, which was suggested to be a result of differing individual neuromuscular responses during the exercise protocol. Previous research has identified a negative correlation between muscle mass and neuromuscular fatigue (Fallowfield, Blacker, Willems, Davey, & Layden, 2012). From this, secondary analysis was conducted to identify if those participants in IMT_{CON}, where no reduction in MVC peak force was observed at any time point (Table 6.7) was related, to an increase in baseline maximum strength within this group. Indeed it was established that participants within this group exerted higher peak forces during preliminary assessments also demonstrated reduced levels of fatigue during MVC conducted post-LC (r= -0.627 , P<0.05, Figure 6.14, panel A) and post-LC_{TT} (r= 0.019, P>0.05, Figure 6.14, panel B) in line with the findings from previous research (Fallowfield et al., 2012). This suggests

that increased muscle mass through regular training may protect against the development of peripheral fatigue of the locomotor musculature and may be an important component of physical preparations for occupational and recreational tasks.

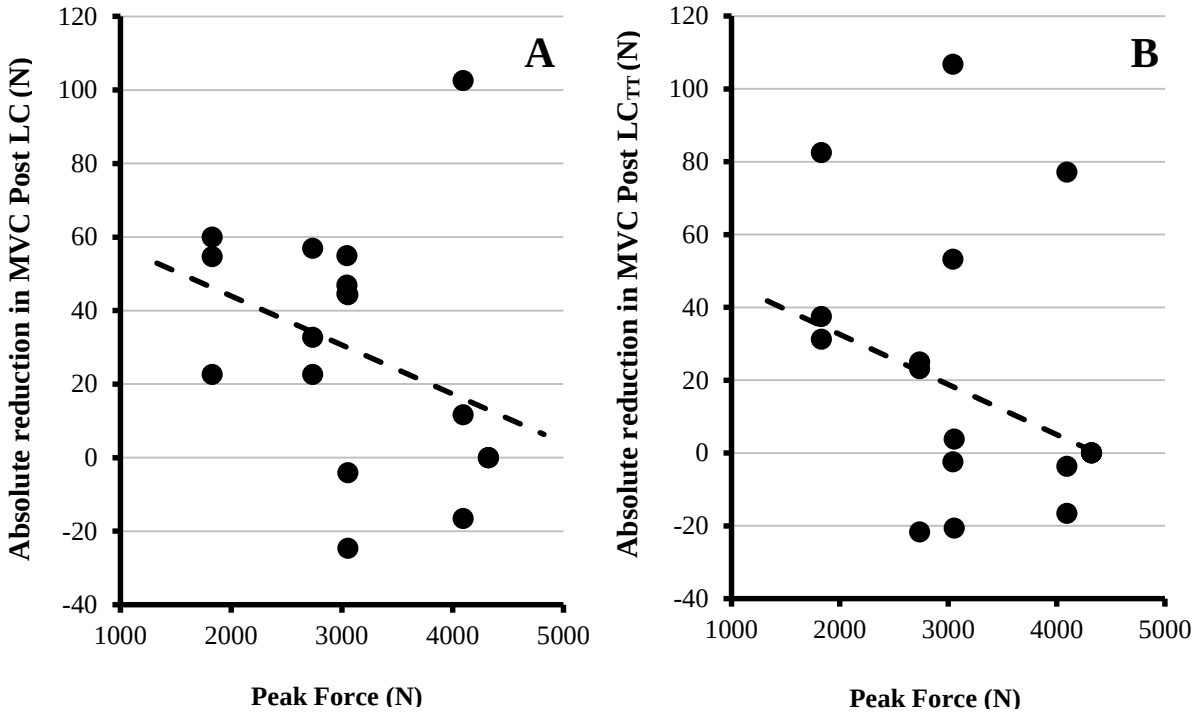

Figure 6.15 Subgroup analysis of IMT_{CON} data demonstrating a negative correlation for absolute reduction in MVC peak force from baseline against maximum strength values obtained during preliminary assessments, Panel A: post-LC (r= -0.650, P<0.05) and B: post LC_{TT} (r= -0.356, P>0.05).

6.5 CONCLUSION

A key theme throughout this thesis is the presence of respiratory muscle fatigue, which may have important consequences for some occupational and recreational activities where thoracic load carriage is a prominent feature. Thoracic load carriage impairs whole body performance during occupationally relevant tasks (Chapters 3). This is due to increases in metabolic (Borghols, Dresen, & Hollander, 1978) and neuromuscular demands (Blacker, Fallowfield, et al., 2010), which pose a critical and real threat to performance via altered respiratory muscle function. Inspiratory muscle training attenuates the negative

cardiorespiratory and perceptual consequences and improves exercise performance with thoracic load carriage as demonstrated for the first time in Chapter 5 and in this study. The improvement is enhanced by incorporating a period of IMT_F, which provides an additional ergogenic effect to 2.4 km time-trial performance that is pre-loaded with 60 min sub-maximal exercise. This appears to be the result of improved coordination between core and respiratory muscle groups that are tasked heavily via load carriage and IMT_F. However, it did not attenuate respiratory muscle or locomotor muscle fatigue observed after load carriage activities.

CHAPTER 7

GENERAL DISCUSSION

7.1 KEY FINDINGS

The aim of this thesis was to investigate the limitations and trainability of the respiratory system during both sub-maximal and maximal exercise with external loads worn upon the thorax and the potential for inspiratory muscle training techniques to overcome such limitations and improve performance. The key findings here demonstrate that:

I) There was a need for an exercise protocol that closely reflects occupational and recreational activities and contains greater ecologically validity than those adopted previously. Here, a protocol was devised containing both sub-maximal and maximal exercise components that considers current military training criteria and can be used to reliably quantify load carriage performance. The intraclass correlation was 0.85, coefficient of variation was 10.5% and Cohen's d was 0.35, demonstrating a very good level of reliability and providing, for the first time, a useful tool for assessment of load carriage activities; which was subsequently used in all chapters of this thesis.

II) Previous research has quantified the physiological effects of exercise with a 25 kg thoracic load, but has not researched it effects on respiratory muscle function. Here, reduced respiratory muscle pressures were observed following 60 minutes of treadmill walking at 6.5 $km \cdot h^{-1}$ (mean reduction ~12%, $P<0.05$), which was further reduced following a 2.4 km self-paced time trial (mean reduction ~15%, $P<0.05$). Load carriage exercise also evoked increased physiological and perceptual responses.

III) Acute inspiratory loading has demonstrated ergogenic benefits to performance in whole body exercise tasks via increased baseline respiratory muscle strength. Traditional whole body warm up techniques were combined with inspiratory warm up techniques at 40% P_{Imax}, providing a transient increase in respiratory muscle strength (~7% $P<0.05$) that did not translate to an improvement in performance on a 2.4 km self-paced time trial.

IV) Chronic inspiratory muscle loading produced a mean decrease of 1.3 ± 0.7 min, 8% (P<0.05) in time trial performance following 6 weeks of training in Chapter 5 and a mean increase of 1.2 ± 0.8 min, 7%, (P<0.05) in time trial performance following four weeks of training in Chapter 6. Post IMT, HR and perceptual responses were reduced following 60 minutes of treadmill marching (P<0.05) in Chapter 5. The principal mechanism for increased performance was greater absolute strength of the inspiratory muscles, which was ~31% greater in Chapter 5, (P<0.05) and ~14% in Chapter 6 (P<0.05). Despite increased performance and P_{Imax}, the relative reduction in P_{Imax} at each time point was unchanged as IMT failed to protect against the onset of respiratory muscle fatigue.

V) Finally, the use of IMT_F techniques which may target the dual relationship of respiratory muscles provides an additional ergogenic effect to exercise performance (4%, P<0.05) and P_{Imax} (7%, P<0.05). The improvements observed here are above those observed in Chapter 5 and also increased core muscle strength and performance on SEPT (18%, P<0.05). Similar to the findings in Chapter 5, IMT and IMT_F failed to protect against respiratory muscle fatigue.

7.2 EFFECTS OF LOAD CARRIAGE UPON RESPIRATORY MUSCLE STRENGTH

Throughout this thesis it has been consistently demonstrated that exercise with load carriage reduces the force generating capacity of the respiratory muscles. Throughout each chapter P_{Imax} was reduced following 60 minutes of load carriage at 6.5 $km \cdot h^{-1}$ relative to resting values (range 11–13%, P<0.05) and again following the 2.4 km time trial relative to post load carriage values (range 3–5%, P<0.05) and resting values (range 13–17%, P<0.05). In comparison, there were no reductions in P_{Imax} during an identical trial without a load (post LC 1%, post LC_{TT} 4%, P>0.05). The exact mechanism is unknown but it is likely to be the result of elevated work of breathing and impaired breathing mechanics imposed by both chest

wall loading and restriction (Dominelli et al., 2012; Tomczak et al., 2011). Exercise with load carriage increases the work of breathing through a curvi-linear increase in the force and velocity of contraction (Brown & McConnell, 2012). This increases the work done by the inspiratory musculature for a given breath; which occurs through changes in EILV and the addition of the restrictive component of the backpack, imposed by the shoulder straps, which also reduces operational lung volumes. In combination, further reductions in the efficiency of the respiratory musculature occurs as their position on their length tension curve becomes suboptimal and subsequently leads to reduced efficiency (Dominelli et al., 2012).

Reductions in P_{Emax} were also present following 60 minutes of load carriage at 6.5 $km \cdot h^{-1}$ relative to baseline values (range 11–15%, $P<0.05$) and further reduced following a 2.4 km time trial relative to post-LC values (range 3–6%, $P<0.05$) and baseline values (range 16 – 18%, $P<0.05$). This is likely caused by the positioning of the load upon the thorax which alters posture and increases core muscle activation to support the position of the load whilst as a compensatory measure for altered centre of mass (Attwells et al., 2006). Also here, the expiratory musculature also work outside of their optimal placement on the length tension curve, due to an imposed reduction in end-expiratory lung volume (Dominelli et al., 2012). This autonomous alteration seeks to reduce the imposed physiological and biomechanical stress on the body during exercise (Knapik et al., 2012). During unloaded running exercise, it has been demonstrated that fatigue of the muscles that comprise the abdominal wall occurs due to their role in controlling the positon of the trunk (Tong, Wu, Nie, et al., 2014), which is further exacerbated in the presence of the load (Al-Khabbaz, Shimada, & Hasegawa, 2008). The dual role of this group during exercise is also a possible consideration for the observed fatigue of the expiratory muscles. During peak ventilation the expiratory muscles are recruited proportionally to assist with expiration and there are considerable increases in breathing pattern, as observed in Chapters 4 and 5. Changes in f_B and V_T occur to facilitate the

increase in $\dot{V}_E$, which are indicative of increased demand for alveolar ventilation and subsequently inspiratory and expiratory recruitment (Sheel & Romer, 2012). The ventilatory demands are exacerbated during exercise with thoracic loads due to an imposed limitation placed upon the thorax causing a global increase in the work of breathing.

7.3 EFFECTS OF LOAD CARRIAGE ON PHYSIOLOGICAL AND PERCEPTUAL RESPONSES

In summary and with the exception of $[lac^-]_B$ and $[glucose^-]_B$ concentrations, all measured physiological variables increased during LC. The finding of similar $[lac]_B$ and $[glucose]_B$ concentrations is in agreement with existing load carriage research that has investigated the physiological consequences of load carriage walking at 6.5 $km \cdot h^{-1}$ (Blacker et al., 2009) and is attributed to exercise intensities that are markedly below lactate threshold. Lactate threshold has been shown to vary between 61% and 81% $\dot{V}O_{2peak}$ in trained individuals (Coyle, 1994) which is above the mean exercise intensity observed during load carriage activities within this thesis (~50% $\dot{V}O_2$ peak). During LC_{TT} however, $[lac^-]_B$ increased significantly (mean increase 6.3 $mmol \cdot l^{-1}$, $P<0.05$). This coincided with changes in exercise intensity that increased from 50% $\dot{V}O_2$ peak to 76% $\dot{V}O_2$ peak, thus breaching lactate threshold and resulting in increased $[lac^-]_B$ accumulation ($P<0.05$). $\dot{V}O_2$ and HR responses to load carriage also increased accordingly with the previous findings of Blacker et al. (2009), and although not reported within the chapters of this thesis, the data supports the suggestion of cardiac and $\dot{V}O_2$ drift that have been previously observed in load carriage activities >60 min (Blacker et al., 2009; Patton et al., 1991). Increased $\dot{V}O_2$ drift occurs as the result of increased O_2 utilisation resulting from increased force production and muscle fibre recruitment from the locomotor and stabilising muscle groups due to the absolute increase in work, increasing O_2 demand (Blacker et al., 2009). The presence of a drift is suggested to occur due to the prolonged nature of load carriage exercise which sees muscle fibres become

fatigued and/or damaged, thus reducing the force they are able to produce (Blacker, Fallowfield, et al., 2010; G. Y. Millet, Martin, Lattier, & Ballay, 2003). To compensate for this, additional motor units are then recruited to maintain movement and this additional recruitment increases O_2 demand therefore driving an upward drift in $\dot{V}O_2$. The results of Chapter 6 suggest this may be a likely mechanism as observable reductions in m. quadricep force were observed at each time point; however, a lack of statistical significance makes comparisons with previous literature difficult.

Consistent changes in perceptual responses were recorded during load carriage activities undertaken within this thesis. Perceptual responses were divided into three categories to allow an element of specificity to differing regions of the body and to identify whole body, locomotor muscle and breathing discomfort. The key mechanism in the modulation of perceived effort from all regions during exercise is the contribution of afferent feedback that originates from type III and IV muscle afferents. This feedback projects to the central nervous system via the dorsal horn, and is used by the sensorimotor cortex to evoke a perceptual response (Dempsey et al., 2014). Increased breathing discomfort observed here can be explained by increase in the work of breathing imposed upon the inspiratory musculature by the presence of the load and the subsequent inefficient placement of this group upon the length tension curve, forcing them to contract outside their optimum range and leads to the development of premature fatigue (Brown & McConnell, 2012). It is suggested that increased limb discomfort, as observed in experimental Chapters 3 and 5, was the consequence of a respiratory muscle metaboreflex, which stimulates a sympathetically mediated redistribution of $\dot{Q}$ away from locomotor muscles in favour of fatiguing contractions of the diaphragm and other accessory musculature (Dempsey et al., 2006; Sheel et al., 2001). The conclusions of Chapter 6 suggest the presence of this sympathetic redistribution is not apparent during load carriage activities. Therefore, it is likely that

increased limb discomfort is the result of the additional load which increases the work of locomotor muscles during movement. As stated in Chapter 6 and this Chapter (Section 7.2) load carriage increases both motor unit recruitment and muscle damage stimulating increased afferent discharge. The findings of this thesis are the first to detail the physiological responses to maximal self-paced exercise with thoracic loads, and in combination with the findings of Section 7.2, specifically highlight the demand imposed upon and the limitations of the respiratory musculature.

7.4 EFFECTS OF RESPIRATORY MUSCLE LOADING

Of the available methods of respiratory muscle loading, pressure threshold loading techniques are favoured; they target both axes of the force-velocity curve, are near flow independent, maintain a greater sense of practicality compared with other methods and have consistently demonstrated a positive benefit to exercise performance. Within literature, the use of acute and chronic loading protocols have been devised and investigated in relation to improving respiratory muscle strength and whole body performance and both will be discussed here.

7.4.1 EFFECTS OF ACUTE INSPIRATORY LOADING ON P_{IMAX}

Chapter 4 investigated the use of acute inspiratory loading upon performance on LC_{TT}. Following 2 x 30 dynamic inspiratory efforts at 40% P_{Imax}, inspiratory strength was transiently increased by 7% when conducted in isolation and also by 6% when preceded by a 10 min warm up at the velocity of lactate turn point. The increases here are comparable to those observed in previous literature (Lin et al., 2007; Lomax et al., 2011) which is attributed to altered central and peripheral neural efficiency. Increased diaphragm motor evoked potential, coincides with increased efficiency in the synchronisation of accessory muscle contractions (Hawkes et al., 2007; Ross et al., 2007), thus permitting the inspiratory

musculature to contract at a lower relative intensity during exercise. This reduction in inspiratory muscle work attenuates the onset of inspiratory muscle fatigue, metabolite accumulation and perceptual responses during sub-maximal and maximal exercise tasks (Lin et al., 2007; Lomax et al., 2011; Tong & Fu, 2006; Volianitis, McConnell, Koutedakis, McNaughton, et al., 2001). These observations were not present in this thesis, which is likely due to the responses being transient and not the result of more robust structural changes that occur following chronic loading protocols. It may also be the consequence of imposed restriction of the load, as discussed at length in Section 1.4.3, which alters breathing mechanics and increases the work of breathing.

7.4.2 CHRONIC EFFECTS ON P_{IMAX}

Chronic loading protocols possess similar training principles to those of other more superficial skeletal muscles that are readily accessible and respond to regular training via structural adaptations (McConnell, 2009). The use of such protocols was used in Chapter 5 and during the first phase in Chapter 6. Chapter 5 was the first instance in which chronic inspiratory loading protocols were used to assess the ergogenic benefit to load carriage performance using pressure threshold load techniques. In Chapter 6, this was used to prepare a strong foundation within the inspiratory musculature prior to $IMT_{F.}$ Both four and six week interventions elicited an increase in P_{Imax} (14% and 31%, $P<0.05$) respectively. Increased inspiratory muscle strength and subsequent structural adaptations are the result of increased diaphragm thickness and oxidative capacity (Akiyama et al., 1996, 1994). In both Chapters 5 and 6, chronic loading failed to protect the inspiratory musculature from inspiratory muscle fatigue, which is a common response to chronic loading strategies within existing literature. The reduction in P_{Imax} from rest observed post-LC and post LC_{TT} in each trial was similar, as detailed in Section 7.2. The exact mechanism for failing to preserve inspiratory muscle

function is not known but it is suggested it could be the result of altered and inefficient placement on the length tension relationship imposed when a load is positioned upon the thorax. Both interventions were also completed in unloaded and unrestricted conditions which are not representative nor do they target the lung volumes imposed during exercise with load carriage. Therefore, despite increased P_{Imax} at each time point in each intervention, it is suggested that chronic loading techniques may indeed target an inappropriate or inadequate proportion of the length tension curve of the inspiratory muscles rendering them susceptible to fatigue during exercise with load carriage.

7.4.3 PHYSIOLOGICAL AND PERCEPTUAL RESPONSES

Acute inspiratory loading had no effect on the physiological and perceptual responses measured in Chapter 4. Following chronic inspiratory loading protocols and during submaximal load carriage exercise, reductions were observed in HR, RPE and RPE_{legs} when compared with pre-intervention values. This is attributed to the increased strength of the inspiratory muscles, which allows them to work at a lower relative intensity during exercise. This has important implications for cardiovascular demand and the discharge of type III and IV muscle afferents, which are important in regulating the physiological responses to exercise, discussed in more detail in Section 7.4.5.

7.4.4 EFFECTS OF RESPIRATORY LOADING ON PERFORMANCE

As discussed above, acute inspiratory loading provides a stimulus specifically to the respiratory muscles and when combined with traditional whole body priming activities in previous literature, provides a transient increase in P_{Imax} and whole body exercise performance. Although IMWU elicited a transient increase in P_{Imax} (7%, $P<0.05$) this did not translate to an increase in exercise performance. This is likely the result of the duration and performance test used, as previous studies that have observed improvements in performance

have employed either open ended fixed intensity exercise or fixed duration all out exercise that is of a short (~10 min) duration (HajGhanbari et al., 2013). This suggests that the transient benefit provided by acute inspiratory loading and the benefit to performance is lost; therefore, the transferable benefit to performance in occupational settings is questionable.

A plethora of research exists detailing the effects of chronic loading strategies and subsequent increases in performance in whole body exercise tasks and reductions in fatigue of the inspiratory muscles (HajGhanbari et al., 2013; Illi et al., 2012). Three key methods of chronic loading exist that target differing aspects of the flow-volume relationship, as detailed in Section 1.3.2. In this thesis, pressure threshold loading techniques were applied to a commonly used protocol that saw 2 x 30 dynamic inspiratory efforts completed with a training load of 50% P_{Imax}. Research illustrates a variable response to exercise performance when adopting chronic loading techniques which are attributed to differing exercise demands, exercise modalities and populations used. Within this thesis, chronic loading was used in Chapter 5 and during phase one of Chapter 6, which was the first instance where this was used in conjunction with load carriage activities. Both six and four week interventions elicited an 8% improvement (P<0.05) in performance on LC_{TT}. The results of Chapter 5 are the first demonstration that IMT has a genuine ergogenic effect upon 25 kg load carriage exercise performance. In summary, the findings suggest that acute inspiratory loading techniques may not be beneficial in providing an ergogenic benefit to performance on LC_{TT}; however, the data here provides convincing evidence that the use of chronic inspiratory loading techniques are due to the structural adaptations that occur in response to chronic loading.

7.4.5 MECHANISMS OF IMPROVEMENT

The causal factor here is improved performance on load carriage tasks as observed in Chapters 5 and 6, and is primarily the result of increased inspiratory muscle strength. The improvements discussed previously permit the inspiratory muscles to work at a lower relative intensity during exercise (Turner et al., 2012). This adaptation is observed via increased P_{Imax} following a period of chronic loading (4 or 6 weeks as detailed previously). This has a multifaceted effect on physiological systems and whole body performance. First, reduced heart rate was observed during load carriage activities, which is characterised by fixed intensity submaximal exercise. Reduced heart rate occurs as a result of diaphragmatic contractions occurring at reduced relative intensities, thus reducing cardiovascular demand during exercise. Second to this, normalised intra-thoracic pressures swings during the breathing cycle, reducing the work of the diaphragm during exercise, thus the respiratory muscles command reduced levels of $\dot{Q}$ (Miller et al., 2002). Third, reduced perceptual responses were also observed following LC in Chapters 5 and 6. The likely mechanism for this reduction is a blunted discharge frequency of mechano-sensitive type III and IV nerve afferents, which project to the sensory cortex (Dempsey et al., 2006). This is caused by reduced metabolite accumulation that stimulate the chemically sensitive afferents and reducing afferent feedback and effort perceptions during exercise tasks (Sinoway et al., 1993).

Chapter 5 hypothesised reductions in cardiovascular strain and perceptual effort may result from an attenuation of the respiratory muscle metaboreflex, which exists to preserve fatiguing contractions of the diaphragm during exercise and exacerbates both of these responses (Dempsey et al., 2006). It is accepted that this may be unlikely as it is expected that O_2 extraction would increase to meet the demand (Bailey et al., 2010). This suggestion was

confirmed in Chapter 6 as there were no reductions in peak force on the isometric voluntary and electrically evoked contractions and suggests that, attenuated physiological responses are most likely, as suggested above, due in part to a reduction in the discharge frequency of group III and IV afferents (Dempsey et al., 2014). This was confirmed in a recent study by Amann et al. (2010) where it was documented that a continuous supply of afferents from working locomotor and respiratory muscle afferents plays a crucial role in regulating the cardiorespiratory responses to exercise. Both of which are important for preventing premature fatigue and ensuring optimal performance.

7.5 FUNCTIONAL IMT, THE FUTURE?

The use of functional inspiratory muscle training techniques was recently proposed by Tong et al., (2014); this technique targets the closely integrated relationship between muscles which comprise the abdominal complex and the muscles of inspiration. During exercise this group is tasked with assisting with ventilation and also, torso and lumbopelvic stiffness to increase stability and optimise the kinetic chain of upper and lower extremities. However, following high-intensity running, reductions in core muscle function suggests the presence of fatigue in this group (Tong, Wu, Nie, et al., 2014). Targeting both inspiratory/core muscle function in a holistic approach has only been demonstrated twice to date. Tong et al. (2014) demonstrated 4% increase in performance in recreational runners, where increased core muscle strength was also improved in the training group only. Here (Chapter 6), 4 wks IMT_F elicited a greater improvement in both P_{Imax} (6%, $P<0.05$), LC_{TT} performance (4%, $P<0.05$) and core muscle strength (18%, $P<0.05$) relative to pre IMT_F values, and no change was observed in a control. Although the initial findings from these studies provides a positive indication to the uses and application of the functional methods, this area is still in its infancy and requires greater research to investigate the application of the findings to different

sports/exercise tasks and to outline which exercises elicit the greatest/most beneficial adaptations. The initial findings are however, positive and the use of such methods may in future studies develop the application of respiratory loading techniques and the impact this has on exercise performance. This could include more sport specific study designs and the use of sport specific exercises to determine the effects this has in differing exercise groups. Strengthening the accessory muscles using traditional strengthening exercises of the accessory muscles to coincide with functional training regimes might also be of interest.

7.6 METHODOLOGICAL LIMITATIONS AND FUTURE DIRECTIONS

A key limitation is the application to occupational groups; this is apparent, as the participants that formed the studies (except for two participants in Chapter 6) had no experience of military training. Careful selection was implemented to ensure that all participants had previous experience with load carriage from recreational activities, which coincided with extensive familiarisation prior to all experimental trials. Consequently, the application of the findings to those in occupational groups remains questionable despite small subgroup analysis (N= 2) of those participants with military backgrounds demonstrating a similar effect to respiratory, physiological and performance parameters. This is not sufficient to make a conclusive statement however, and therefore warrants further investigation in occupational groups.

A further limitation is the study design of Chapter 3, which attempted to investigate the effects of thoracic load carriage on respiratory muscle function. Fatigue was observed following LC and LC_{TT} when compared with a control but the extent to which thoracic restriction reduces performance was not measured. It is suggested in the discussions of Chapters 3, 5 and 6 that thoracic restriction is a potential mechanism for the observed fatigue via reductions in P_{Imax}, which is proposed from the findings of previous literature that has

identified reductions in P_{di} when inelastic strapping reduced TLC by 33-40% and also reduced exercise performance (Miller et al., 2002; Tomczak et al., 2011). Future research should seek to investigate and quantify reductions in P_{Imax} and/or performance on the load carriage tasks utilised here in response to thoracic restriction. Furthermore, the use of volitional mouth pressures to determine respiratory muscle strength and fatigue post exercise have been criticised. Volitional measures of respiratory muscle force do not directly reflect solely the force of the diaphragm; instead P_{Imax} reflects the volitional force of all inspiratory muscles working in synergy and may not be sufficiently sensitive to rule out accessory muscle fatigue, the influence of motivation, central fatigue and other factors. However, P_{Imax} and P_{di} are correlated before and after IMT (Brown et al., 2014); therefore it can be suggested that measures of ΔP_{Imax} provides a useful measure of inspiratory muscle force and a useful surrogate measure of diaphragm function. It is acknowledged that non-volitional measures of muscle force are preferred by measuring P_{ga}, P_{oes} and hence P_{di} in response to electrical or magnetic stimulation. Although the technical limitations surrounding the use of volitional measures are detailed in Section 2.3, considerable time was spent with participants ensuring that familiarisation with this measurement was adequate and motivation was maximised throughout all efforts. It is also important to note here that volitional measures have demonstrated superior between day (i.e., pre to post intervention) reproducibility (Romer & McConnell, 2004). Future research should investigate the effects of load carriage activities, as detailed throughout this thesis, whilst employing non-volitional techniques to specifically assess whether the levels of fatigue observed here are specific to the diaphragm or whether the reduction in force observed is indeed a contribution from all muscles involved in maximal pressure development.

The exercise tasks employed here were laboratory treadmill tests. The design of the exercise protocol detailed in Section 2.10.3.2.2 more closely reflects the physiological strain

and intensity during current occupational training activities, and incorporates both submaximal and maximal exercise tasks in comparison with previously adopted protocols, which lack ecological validity. Although the studies provide an empirical understanding that has furthered the understanding of the physiological consequences of exercise with thoracic loads, it is not wholly representative of either exercise tasks completed or the environmental conditions encountered whilst on deployment on occupational settings. These may otherwise alter the observed physiological responses, thus potentially exacerbating the findings observed here. Additional research that encompasses field based and occupationally relevant exercise tasks is required to further extend the existing knowledge of the physiological demands.

Chapter 6 used non-volitional electrically evoked stimulation of the m. quadricep to quantify the reduction in force production in this group following load carriage exercise. Attention must be paid to the timings and organisation of the stimulation protocol used here and derived from existing research (Blacker, Fallowfield, & Willems, 2013) as described in Section 6.4.4. MVC force remained unchanged despite reductions in voluntary activation, and the findings here do not support previous findings as suggested by Blacker et al. (2010). Therefore, proposing that this could be due to a intramuscular potentiation effect which can last from 1 to 10 mins and is caused by previous maximal contractile activity (Sheel & Romer, 2012). The protocol exhibits intra and inter-day reliability as described in Section 6.2.3.3, but was conducted in the absence of fatiguing exercise where the potentiation effect is likely to be exacerbated. The addition of EMG techniques here would confirm the presence of a potentiation effect and the extent to which muscular force is altered. The observed reductions in voluntary activation are indicative of a reduction in central processes, which existing literature suggests is caused by reduced central motor drive. The stimulation techniques utilised saw electrical currents delivered direct to the muscle belly, which

propagates proximally along the CNS. Although reduced central motor drive is a plausible explanation, it could be the result of a reduction in a number of central processes that the techniques employed here failed to identify. It is however, possible to directly pinpoint the origin of central fatigue via stimulation of different components along the central pathway using transcranial, bi-lateral phrenic nerve stimulation and corticospinal stimulation techniques, which can allow direct investigation and identification of the origin and effects of altered central processes with load carriage activities.

In forming the conclusions for the findings in Chapter 5, it was postulated that the presence of a metaboreflex may be responsible for the reduction in whole body performance. Although this was refuted indirectly in Chapter 6 where the use of electrical stimulation demonstrated no change in force parameters and indicated the presence of a metaboreflex was unlikely despite respiratory muscle fatigue. The use of more objective measures used previously within research; such as ultrasound and thermodilution techniques, may be more appropriate in determining limb vascular resistance, $\dot{Q}$ and during fatiguing work of respiratory and locomotor muscles (Dempsey et al., 2006; Sheel et al., 2001) and should be considered during future investigations that seek to understand the consequences of high levels of respiratory muscle work during load carriage activities.

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APPENDICES

APPENDIX 1

Standard temperature pressure dry conversion table

APPENDIX 2

Reference equations for the determination of gas exchange parameters

APPENDIX 3

Training diary to record IMT adherence

Appendix 4

Reliability data for differing procedures for determining maximal stimulation intensity used during Chapter 6.

Appendix 5

Brown, P, I., Venables, H, K., Liu, H.., de-Witt, J, T**., Faghy, M, A.,** (2013), Ventilatory muscle strength, diaphragm thickness and pulmonary function in world-class powerlifters., *European Journal of Applied Physiology*, Vol 113(11), pp. 2849-55.

Appendix 6

Faghy, M, A., Brown, P, I., (2014), Preloaded time trial to assess load carriage performance, *Journal of Strength and Conditioning Research*, Vol 28(12), pp. 3354-62.

Appendix 7

Faghy, M, A., Brown, P, I., (2014), Thoracic load carriage induced respiratory muscle fatigue, *European Journal of Applied Physiology*, Vol 114(5), pp. 1085-93.

Appendix 8

Faghy, M, A., Brown, P, I., (2015), Training the inspiratory muscles improves running performance when carrying a 25 kg thoracic load in a backpack, *European Journal of Sport Science*. Vol 14, pp.1-10

APPENDIX 1

APPENDIX 1 STANDARD TEMPERATURE PRESSURE DRY CONVERSION TABLE

P_B / mm Hg													
T (°C)	16	17	18	19	20	21	22	23	24	25	26	27	28
740	0.900	.896	.892	.887	.883	.878	.874	.869	.864	.860	.855	.850	.845
742	.903	.898	.894	.890	.885	.881	.876	.871	.867	.862	.857	.852	.847
744	.906	.901	.897	.892	.888	.883	.878	.874	.869	.864	.859	.855	.850
746	.908	.903	.899	.895	.890	.886	.881	.876	.872	.867	.862	.857	.852
748	.910	.906	.901	.897	.892	.888	.883	.879	.874	.869	.864	.860	.854
750	.913	.908	.904	.900	.895	.890	.886	.881	.876	.872	.867	.862	.857
752	.915	.911	.906	.902	.897	.893	.888	.883	.879	.874	.869	.864	.859
754	.918	.913	.909	.904	.900	.895	.891	.886	.881	.876	.872	.867	.862
756	.920	.916	.911	.907	.902	.898	.893	.888	.883	.879	.874	.869	.864
758	.923	.918	.914	.909	.905	.900	.896	.891	.886	.881	.876	.872	.866
760	.925	.921	.916	.912	.907	.902	.898	.893	.888	.883	.879	.874	.869
762	.928	.923	.919	.914	.910	.905	.900	.896	.891	.886	.881	.876	.871
764	.930	.926	.921	.916	.912	.907	.903	.898	.893	.889	.884	.879	.874

Note: Slight differences occur among tables and equations due to rounding of temperatures and water vapor pressures.

Derived from Eston & Reilly, (2009)

APPENDIX 2

Calculating the total volume of air expired per minute ('minute ventilation')

$$\dot{\boldsymbol{V}}_{\boldsymbol{E}} = \frac{\boldsymbol{Total\ volume\ of\ expired\ sample\ (L)}}{\boldsymbol{Sample\ duration\ (min)}}$$

Calculating the total volume of air inspired per minute

The Haldane transformation can be used to calculate the *true* inspired volume:

$$\dot{\boldsymbol{V}}_{\boldsymbol{I}} \times \boldsymbol{F}_{\boldsymbol{I}}\boldsymbol{N}_{\boldsymbol{2}} = \dot{\boldsymbol{V}}_{\boldsymbol{E}} \times \boldsymbol{F}_{\boldsymbol{E}}\boldsymbol{N}_{\boldsymbol{2}}$$

This means that the total volume of air inspired can be obtained using the following equation:

$$\dot{\boldsymbol{V}}_{\boldsymbol{I}} = \frac{\dot{\boldsymbol{V}}_{\boldsymbol{E}} \times \boldsymbol{F}_{\boldsymbol{E}}\boldsymbol{N}_{\boldsymbol{2}}}{\boldsymbol{F}_{\boldsymbol{I}}\boldsymbol{N}_{\boldsymbol{2}}}$$

Calculating the volume of oxygen consumed per minute

The volume of oxygen consumed (VO_2) is calculated by subtracting the volume of oxygen expired (V_E x F_EO_2) from the volume of oxygen inspired (V_I x F_IO_2):

$$\dot{\boldsymbol{V}}\boldsymbol{O}_{\boldsymbol{2}} = \left(\dot{\boldsymbol{V}}_{\boldsymbol{I}} \times \boldsymbol{F}_{\boldsymbol{I}}\boldsymbol{O}_{\boldsymbol{2}}\right) - \left(\dot{\boldsymbol{V}}_{\boldsymbol{E}} \times \boldsymbol{F}_{\boldsymbol{E}}\boldsymbol{O}_{\boldsymbol{2}}\right)$$

$$\dot{\boldsymbol{V}}\boldsymbol{C}\boldsymbol{O}_{\boldsymbol{2}} = \left(\dot{\boldsymbol{V}}_{\boldsymbol{E}} \times \boldsymbol{F}_{\boldsymbol{E}}\boldsymbol{C}\boldsymbol{O}_{\boldsymbol{2}}\right) - \left(\dot{\boldsymbol{V}}_{\boldsymbol{I}} \times \boldsymbol{F}_{\boldsymbol{I}}\boldsymbol{C}\boldsymbol{O}_{\boldsymbol{2}}\right)$$

Calculating the 'Respiratory Exchange Ratio' (RER) and energy expenditure

$$\boldsymbol{RER} = \frac{\dot{\boldsymbol{V}}\boldsymbol{C}\boldsymbol{O}_{\boldsymbol{2}}}{\dot{\boldsymbol{V}}\boldsymbol{O}_{\boldsymbol{2}}}$$

APPENDIX 3

Name:	Week Number:	Week Starting:

Monday		Tuesday		Wednesday		Thursday	
Session 1	Session 2	Session 1	Session 2	Session 1	Session 2	Session 1	Session 2
Own Training		Own Training		Own Training		Own Training	
Friday		**Saturday**		**Sunday**		**Notes**	
Session 1	Session 2	Session 1	Session 2	Session 1	Session 2	Procedure: • 2 x 30 breathes • 25-30 should be hard breathes • Please denote with a "" if you have increased the tension spring Other Comments:	
Own Training		Own Training		Own Training			

If you have any problems/ questions please contact: Mark Faghy (tel: 07986 322 947, e-mail: m.faghy@derby.ac.uk)

APPENDIX 4

STIMULATION OUTPUT DETERMINED SEPARATELY UPON EACH VISIT

	Day One				Day Two				Day Three				Between Day Reliability	
	Pre-LC	**Post-LC**	**Post-LC$_{TT}$**	**Post-30**	**Pre-LC**	**Post-LC**	**Post-LC$_{TT}$**	**Post-30**	**Pre-LC**	**Post-LC**	**Post-LC$_{TT}$**	**Post-30**	***P***	**95% LoA**
MVCPeak Force (N)	654 ±117	632 ± 123	624 ± 96	616 ± 95	594 ± 110	625 ± 87	610 ± 95	612 ± 128	594 ± 110	625 ± 87	610 ± 95	612 ± 128	**0.660**	**41.5**
VA (%)	70 ± 34	61 ± 33	76 ± 42	68 ± 47	57 ± 28	46 ± 29	50 ± 39	48 ± 31	53 ± 31	45 ± 34	48 ± 40	45 ± 33	**0.490**	**829.7**
Twitch Peak Force (N)	170 ± 47	172 ± 59	157 ± 51	153 ± 42	179 ± 57	188 ± 54	189 ± 66	188 ± 53	178 ± 47	198 ± 67	174 ± 48	188 ± 67	**0.001**	**96.5**
Doublet Peak Force (N)	386 ± 90	395 ± 89	366 ± 89	374 ± 107	372 ± 116	403 ± 87	393 ± 88	392 ± 99	371 ± 84	422 ± 108	411 ± 94	414 ± 84	**0.144**	**59.5**
20 Hz Peak Force (N)	285 ± 84	280 ± 85	271 ± 76	250 ± 66	308 ± 96	311 ± 86	303 ± 102	346 ± 139	314 ± 97	363 ± 119	352 ± 97	340 ± 98	**0.001**	**162.9**
50Hz Peak Force (N)	368 ± 124	376 ± 102	361 ± 74	351 ± 109	398 ± 131	414 ± 131	424 ± 187	441 ± 165	415 ± 149	482 ± 159	482 ± 139	499 ± 138	**0.000**	**180.9**
20:50 Hz Ratio	0.74 ± 0.10	0.75 ± 0.10	0.75 ± 0.11	0.72 ± 0.09	0.72 ± 0.13	0.75 ± 0.11	0.75 ± 0.13	0.73 ± 0.08	0.76 ± 0.07	0.76 ± 0.07	0.73 ± 0.07	0.69 ± 0.10	**0.623**	**30.5**

PRE-DETERMINED STIMULATION INTENSITY DETERMINED UPON FIRST VISIT

	Day One				Day Two				Day Three				Between Day Reliability	
	Pre-LC	**Post-LC**	**Post-LC$_{TT}$**	**Post-30**	**Pre-LC**	**Post-LC**	**Post-LC$_{TT}$**	**Post-30**	**Pre-LC**	**Post-LC**	**Post-LC$_{TT}$**	**Post-30**	***P***	**95% LoA**
MVCPeak Force (N)	535 ± 98	526 ± 74	530 ± 68	529 ± 71	526 ± 85	532 ± 82	531 ± 62	538 ± 75	531 ± 91	531 ± 100	538 ± 100	530 ± 96	0.805	19.2
VA (%)	64 ± 34	67 ± 31	58 ± 31	62 ± 32	60 ± 34	62 ± 37	65 ± 41	66 ± 39	62 ± 463	73 ± 44	69 ± 37	62 ± 37	0.766	461.1
Twitch Peak Force (N)	113 ± 24	115 ± 29	113 ± 26	114 ± 21	117 ± 21	115 ± 24	112 ± 25	1112 ± 24	115 ± 27	113 ± 27	113 ± 26	117 ± 25	0.556	28.0
Doublet Peak Force (N)	298 ± 44	299 ± 46	296 ± 48	302± 51	300 ± 38	307 ± 51	304 ± 70	299 ± 50	303 ± 72	301 ± 54	301 ± 46	300 ± 34	0.654	31.7
20 Hz Peak Force (N)	302 ± 73	295 ± 77	301± 80	301 ± 74	300 ± 67	304 ± 87	305± 90	299 ± 93	303 ± 88	300 ± 93	300 ± 91	300 ± 92	0.420	33.4
50Hz Peak Force (N)	379 ± 51	390 ± 74	402 ± 67	386 ± 79	392 ± 91	399 ± 91	403 ± 84	397 ± 96	399 ± 95	402 ± 88	398 ± 88	405 ± 759	0.610	33.5
20:50 Hz Ratio	0.80 ± 0.07	0.79 ± 0.07	0.78 ± 0.07	0.77 ± 0.06	0.80 ± 0.07	0.80 ± 0.07	0.79 ± 0.05	0.79 ± 0.07	0.80 ± 0.07	0.78 ± 0.08	0.77 ± 0.06	0.77 ± 0.06	0.817	30.5